

QUANTITATIVE STUDIES ON THE DEVELOPMENT AND CONTROL
OF BLACK SHANK OF TOBACCO, AND ON THE SURVIVAL OF
PHYTOPHTHORA PARASITICA VAR. NICOTIANAE IN SOIL

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1979

ACKNOWLEDGMENTS

The author wishes to express her appreciation and gratitude to Dr. David J. Mitchell, with whom she had the pleasure of working, for his time, interest, support, and guidance during the course of her studies. She would also like to thank Dr. Norman C. Schenck for assistance that made portions of this research possible.

Gratitude is given to the members of her supervisory committee for their assistance during the preparation of this manuscript, and to Dr. G. E. Sanden for his co-operation in the field studies conducted. She would also like to thank her friends and co-workers, James and Eric, for their thoughtfulness, patience, assistance, and humor.

Special thanks are extended to her parents for their concern and assistance, and to her friends who gave support, love, and strength.

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Abstract of Dissertation Presented to the Graduate
Council of the University of Florida in Partial
Fulfillment of the Requirements for the
Degree of Doctor of Philosophy

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March, 1979

Chairman: David J. Mitchell
Major Department: Plant Pathology

Sonicated chlamydospores of Phytophthora parasitica var. nicotianae were mixed into autoclaved Astatula sand to achieve defined inoculum densities. Individual 4-wk-old susceptible tobacco plants were exposed to infested soil. After 50 dy at 25 C in a growth room, the percentages of infection were 23, 27, 50, 81, 92, 98, and 100 at 50, 75, 100, 250, 500, 1,000, and 5,000 chlamydospores per kilogram of soil, respectively. Motile zoospores were added to individual plants growing in flooded sand. After 28 dy at 25 C in a growth room, the percentages of infection were 10, 22, 35, 55, 82, 93, and 100 at 5, 15, 25, 50, 100, 200, and 300 zoospores per plant, respectively. Sonicated oospores that were frozen and either

left untreated or treated with cellulase or Gluculase enzymes were mixed into autoclaved soil at densities of 500 to 100,000 spores per kilogram of soil, and were plated on various media to determine germination percentages. None of the enzyme-treated or nontreated oospores germinated after 96-128 hr at 25 C and 12 hr light on various media and none of the tobacco plants were infected after exposure to infested soil for 50 dy at 25 C in a growth room.

Inoculum densities near 0.5 propagules of P. parasitica var. nicotianae per gram of soil resulted in 93 to 100% mortality of susceptible tobacco plants and apparent infection rates (r) of 0.08 to 0.11/unit/dy in the growth room, greenhouse, or field. No black shank occurred under growth-room conditions with 1 or more mg/kg (ppm, w/w) of an experimental fungicide CGA 48988 (CIBA-GEIGY Corp.), incorporated into soil containing 0.5 propagules per gram of soil; under greenhouse conditions 93, 60, 7, and 0% of the susceptible tobacco plants died at 0.5 propagules per gram of soil plus 0, 1, 10, or 50 ppm of the fungicide, respectively. The addition of 130 ml of a 150 ppm solution of the fungicide to susceptible transplants in the field at planting delayed pathogenesis, but after 50 dy it progressed more rapidly ($r=0.22$ /unit/dy) than in nontreated plants ($r=0.10$ /unit/dy). All of the nontreated and treated

susceptible plants and 61 and 10% of the nontreated and fungicide-treated resistant plants, respectively, were dead at harvest. Fungicide-treated resistant and susceptible plants had significantly ($P=0.05$) greater yields than nontreated plants.

Populations of P. parasitica var. nicotianae were monitored over 90 dy in artificially and naturally infested soil that had been maintained at 10 or 25 C and at 6.8% saturation, 27% saturation, or had fluctuated between 27 and 6.8% saturation. Populations were lower than expected in autoclaved soils immediately following infestation, but populations generally increased within 15 dy at 25 C. Populations fluctuated less in naturally infested soil than in artificially infested soil. Populations in all soils decreased after 30-60 dy. The highest populations after 90 dy were in artificially infested soils maintained at 10 C. Survival was poorest in artificially infested raw soil. Populations of the fungus also were monitored in two fields by sampling the former root zones of tobacco plants, or by sampling areas of the field that were disked and left fallow after a growing season in which severe black shank had occurred. All populations in the former root zones and in fallow areas of the field declined during the fall, but ranged from 0.7 to 27 propagules per gram of soil at the end of sampling (11-14 mo after harvest).

SECTION I
RELATIONSHIPS OF NUMBERS OF CHLAMYDOSPORES, OOSPORES,
AND ZOOSPORES OF PHYTOPHTHORA PARASITICA VAR.
NICOTIANAE TO INFECTION AND MORTALITY
OF TOBACCO

Introduction

Phytophthora parasitica Dast. var. nicotianae (Breda de Haan) Tucker [= P. nicotianae Breda de Haan var. nicotianae Waterhouse], the causal agent of black shank of tobacco (Nicotiana tabacum L.), produces several types of spores. Quantitative information about the roles that zoospores, chlamydospores, and oospores of P. parasitica var. nicotianae play in infection and subsequent disease development is limited (20, 26).

Although it was proposed that zoospores of P. parasitica var. nicotianae were the primary inoculum in the field because they were the most frequently observed type of spore (7, 14), there is little evidence to substantiate this claim. Recent studies indicate that zoospores of species of Phytophthora and Pythium may not be the primary source of inoculum (6, 9, 26, 31), but they are considered to be important in the secondary spread of disease in the field (26, 31). Gooding and Lucas (13) showed that disease increased under laboratory conditions

with an increase in zoospore inoculum concentration, but² that disease indices (based on the number of plants killed and the rate of death) at a given inoculum concentration varied significantly in different experiments. Since zoospores must be close to a plant for chemotaxis and infection to occur, their inoculation technique, in which zoospores were injected into sand around plant roots, may have reduced the contact of motile zoospores with roots and thus may have been responsible for the inconsistencies in the disease development observed. Recent studies where motile zoospores were added to the standing water over flooded soil (or vermiculite), which was drained after several hours down through the soil, showed that low numbers of zoospores per plant could result in higher percentages of plant infection and mortality (31, 32). Conditions that favor zoospore motility, i.e., careful handling to reduce encystment and soil moisture conditions that provide water filled channels through which zoospores can move, result in greater infection of plants at a given zoospore concentration than do conditions that induce zoospore encystment or reduce active movement of zoospores (9, 18, 25, 31, 32). Consequently, it is very important to consider the inoculation technique used when disease or inoculum is evaluated quantitatively.

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Chlamydospores of many species of Phytophthora serve as survival structures in soil or in plant debris and have been considered to be the primary inoculum in nature (6, 18, 21, 26, 31, 35). It was reported previously that an inoculum density of 500 chlamydospores of P. parasitica var. nicotianae per kilogram of soil resulted in 93% mortality of susceptible tobacco plants and an apparent infection rate of 0.9/unit/dy under growth-room and greenhouse conditions (20). The numbers of chlamydospores required for specific amounts of infection and mortality of tobacco (in particular, the inoculum densities required for 10, 50, and 90% infection and mortality) and the slope of the linear regression equation for the log-log transformation of inoculum density to disease incidence have not been reported.

Nothing is known about the role of oospores of P. parasitica var. nicotianae in the development of black shank (26). Oospores of P. parasitica var. nicotianae and other heterothallic Phytophthora spp. are formed in abundance only in paired cultures of isolates of opposite compatibility types, but little is known about their role in pathogenesis under natural conditions. The oospores from crosses rarely germinate in vitro (usually 0-2% germination), and thus it is difficult to study the genetics of these fungi, determine the possible pathogenic variability of Phytophthora spp., or learn the

role of oospores in the various cycles of diseases caused by these fungi. There are no known reports of oospores of P. parasitica var. nicotianae in nature (26). Pathogenicity studies have been conducted with oospores of two homothallic Phytophthora spp. [(3), and Baumer and Erwin, unpublished]. Banihashemi and Mitchell (3) found that a density of 5×10^7 oospores of P. cactorum per kilogram of soil resulted in 100% infection of safflower seedlings; Baumer and Erwin (unpublished), however, reported that 20 oospores of P. megasperma per kilogram of soil resulted in 50% mortality of alfalfa seedlings, but that death occurred sooner at higher inoculum levels.

The objective of this study was to determine the relationships of numbers of chlamydospores, oospores, and zoospores to the percentages of infection and mortality of tobacco plants.

Materials and Methods

The isolates of P. parasitica var. nicotianae used in chlamydospore and zoospore studies (P-230) and in oospore studies (P-230 and P-583) were originally isolated from tobacco and obtained from the collection of Phytophthora spp. maintained at the Department of Plant Pathology, University of California, Riverside. The cultures were maintained on V-8 Juice agar and transferred monthly.

Chlamydospores were produced in liquid culture by the method of Tsao (45). Known numbers of chlamydospores, free of viable hyphal fragments and other spores, were obtained as described by Ramirez and Mitchell (35). The infested-soil-layer technique used by Mitchell (30) was modified to allow uninjured tobacco roots to grow into infested soil. Sixty-five grams of Astatula sand that had been autoclaved for 2 hr on two successive days were infested with 0, 50, 75, 100, 250, 500, 5,000, or 50,000 chlamydospores per kilogram of soil, and layered over 15 g of autoclaved builder's sand in a 100-ml polypropylene beaker. The infested soil was covered with 35 g of noninfested soil, and a 1-mo-old tobacco seedling of the susceptible cultivar Hicks was transplanted into the noninfested soil. Plants were maintained in a plant growth room at 25 C and 12 hr light (4,000 lx at the level of the plants) and were watered daily and fertilized

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weekly as described previously (20). Plants were observed daily for wilting and blackening of the stems. Experiments consisted of 15 or 30 plants per inoculum level, and the experiments were conducted six times.

Oospores of P. parasitica var. nicotianae were produced in V-8 Juice broth by the method developed by Honour and Tsao (17). A paired culture of isolate P-230 (A_1 compatibility type) and P-583 (A_2 compatibility type) was initiated by adding 1 ml of a suspension of mycelium of each isolate to 15 ml of V-8 Juice broth in a 200-ml prescription bottle and incubating the cultures for 14 dy in the dark at 25 C. To obtain oospores free of viable hyphal fragments, 2-wk-old cultures were washed and suspended in a $10^{-3}M$ solution of 2-(N-morpholino)-ethanesulfonic acid in deionized water (adjusted to pH 6.2 with 1 N KOH) (MES), comminuted, homogenized, and subjected to sonication (4, 35, 39). The resulting suspension was left undisturbed for approximately 1 hr to allow the spores to settle, and the suspended mycelial debris was discarded. The spores and remaining debris then were resuspended in a larger volume of buffer, the spores again were allowed to settle, and the debris was removed. This process was repeated four times, and the final spore suspension was subjected to low speed centrifugation. The pellet containing the spores was placed in 5-10 ml of buffer and agitated to resuspend the spores, and was

frozen at -20 C to eliminate other viable spores (3, 4⁷). The spore preparation was thawed after 18-24 hr, and 1-ml samples were removed and either diluted with cellulase [a 1% solution of Cellulysin in MES (1:1, v/v) (Calbiochem, San Diego, CA)]or diluted (1:1 or 1:2, v/v) with 2% Gluculase (a mixture containing 163,604 units glucuronidase and 14,995 units sulfatase per milliliter, Endo Laboratories, Inc., Garden City, NY). After 24-72 hr incubation at 25 C in the dark, enzyme-treated spores were washed, suspended in the buffer, and concentrated four times using low-speed centrifugation to remove the enzymes from the spore preparations. Soil was infested with 0, 500, 5,000, 50,000, or 100,000 oospores per kilogram, and plants were transplanted and maintained as described for plants in the chlamydospore experiments.

Zoospores were produced in cultures initiated by placing six 2-mm plugs from the margin of a 2-dy-old culture of P. parasitica var. nicotianae on V-8 Juice agar in petri plates containing 15 ml of V-8 Juice broth. Cultures were incubated at 25 C and 12 hr light (4,000 lx at the level of the cultures). The medium was drained from the plates after 48 hr and each mycelial mat was washed three times with 25 ml of an autoclaved solution of 10^{-4} M MES (pH 6.2) and flooded with 10 ml of the buffer. After incubation for an additional 48 hr to induce sporangium formation, the cultures were rinsed

once and flooded with 25 ml of the buffer and chilled in a refrigerator at 3 C for 15 min. Zoospores were released 10-30 min after the cultures had been returned to room temperature. The zoospore suspension from the plates was diluted 1:1 with the buffer, and 3-ml samples were removed and agitated on a vortex mixer to induce spore encystment (42). The encysted spores were counted using the hemacytometer and micro-drop methods described by Mitchell et al. (32). The zoospore suspension then was diluted with the buffer to give 50 zoospores per milliliter (one zoospore per 20- μ l drop), and the motility of zoospores was checked by observing spores in six 20- μ l drops. The procedures for inoculation of plants with zoospores were designed to simulate flooded conditions in the field where zoospores move in the surface water to stems or are carried to the roots as infested water drains through the soil. Individual 1-mo-old Hicks plants were transplanted into 60 g (30 ml volume) of washed, autoclaved builder's sand in 50-ml polypropylene beakers. The beakers had small holes in the bottom to allow water movement. Beakers were placed in nylon trays (15 per tray) and were maintained in a growth room at 25 C and 12 hr light (4,000 lx at the level of the plants) for 1-2 wk prior to inoculation. Plants were watered daily and fertilized once a week with modified Hoagland's solution (15). Immediately before inoculation the trays were filled with tap

water to a level that provided 1 cm of standing water above the surface of the sand in the beakers. Five, 15, 25, 50, 100, 200, or 300 zoospores were added to each of 15 beakers per tray by pipetting 0.1, 0.3, 0.5, 1.0, 2.0, 4.0, or 6.0 ml of a zoospore suspension that contained 50 zoospores per milliliter into the surface water of each beaker. Experiments consisted of two trays of each treatment at each inoculum level. After 3 hr the water was drained from the trays which allowed the zoospore-infested water to move down through the sand in the beakers. Plants were maintained in the growth room for 14-28 dy.

The percentages of germination of chlamydospores, oospores, or zoospores from inocula or infested soil were determined by plating samples on a selective medium (PARP) which contained the following ingredients: 10 mg pimäricin (Delvocid, 50% ai, Gist-Brocades N.V., Delft, Holland), 250 mg ampicillin (Polycillin-N, 81% ai, Bristol-Meyers Co., Syracuse, NY 13201), 10 mg rifampicin (Rifamycin SV, 100% ai, Sigma Chemical Co., St. Louis, MO 63178), 100 mg pentachloronitrobenzene (Terraclor, 75% ai, Olin Mathieson Chemical Corp., Little Rock, AR 72203), and 17 g Difco cornmeal agar in 1.0 liter of deionized water. The plates were incubated at 25 C in the dark for 24-48 hr and observed for colony development. Oospore suspensions also were plated on cornmeal agar, noble agar, Difco agar, V-8 Juice agar, and the PVP medium of

Tsao and Ocaña (46), and incubated at 25 C with 12 hr of light (4,000 lx at the level of the plates) for 96-148 hr.

Plants in all experiments were observed daily and considered dead when they had wilted permanently and had blackened stems. The roots and stems of surviving plants that had been inoculated with chlamydospores, oospores, or zoospores were washed in running water, dipped in 70 % ethyl alcohol for 15 sec, rinsed twice in deionized water, and blotted dry on paper towels. Plants were plated on the selective medium (PARP), and incubated for 48 hr at 25 C in the dark; the plates then were observed for the growth of P. parasitica var. nicotianae from the stems and roots of infected plants.

Results

The percentages of infection and mortality of tobacco increased when the number of chlamydospores of P. parasitica var. nicotianae (Fig. 1) to which the plants were exposed increased from 50 to 5,000 chlamydospores per kilogram of soil. After 50 dy, 23 and 100% of the plants were infected at 50 and 5,000 chlamydospores per kilogram of soil, respectively. The infection data were transformed to $\log_e 1/(1-x)$, where x equals the proportion of the plants infected, to adjust for multiple infections (48). When $\log_{10}(\log_e 1/(1-x))$ was plotted against $\log_{10}$ of the number of chlamydospores per kilogram of soil (Fig. 2), points of the log-log transformation plot lay in a straight line between 50 and 1,000 chlamydospores per kilogram of soil. The slope of the line determined by linear regression analysis was 0.93 ($P=0.001$). The inoculum density required for 50% infection of the plants was interpolated to be 132 chlamydospores per kilogram of soil. Data presented are from experiments conducted in autoclaved Astatula sand, but similar levels of infection were obtained at the corresponding inoculum levels in raw or methyl bromide-treated Astatula sand.

Recovery of the fungus from chlamydospore-infested soil increased from 30-40% when the soil was plated

immediately following infestation to 70-90 % when the soil was plated one day after infestation.

The percentages of infected tobacco plants in sand increased from 5 to 200 zoospores per plant (Fig. 3). When data were transformed to $\text{Log}_e 1/(1-x)$ to compensate for multiple infections and plotted as $\text{Log}_{10}(\text{Log}_e 1/(1-x))$ against Log_{10} of the number of zoospores per plant, points obtained at 5 to 200 zoospores per plant lay in a straight line (Fig. 4). The slope of the line was determined to be 0.91 ($P=0.001$) using linear regression analysis and the inoculum level required for 50% infection was interpolated to be approximately 42 zoospores per plant. Greater than 70% of the zoospores were motile at the time of inoculation of the plants, and 70-90% of the zoospores germinated when samples of the inoculum were plated on the selective medium.

With few exceptions, all of the infected plants died within 14 or 50 dy after the initiation of the zoospore or chlamydospore experiments, respectively. None of the plants in the oospore experiments died or were infected, as determined by plating on the selective medium, within 50 dy after they were transplanted into beakers containing infested soil. None of the enzyme-treated or nontreated oospores of P. parasitica var.

nicotianae germinated on cornmeal agar, Difco agar,

noble agar, V-8 Juice agar, PARP medium, or PVP

medium.

Fig. 1. The relationship of density of chlamydospores of *Phytophthora parasitica* var. *nicotianae* in soil to percentage infection of tobacco plants 50 dy after planting.

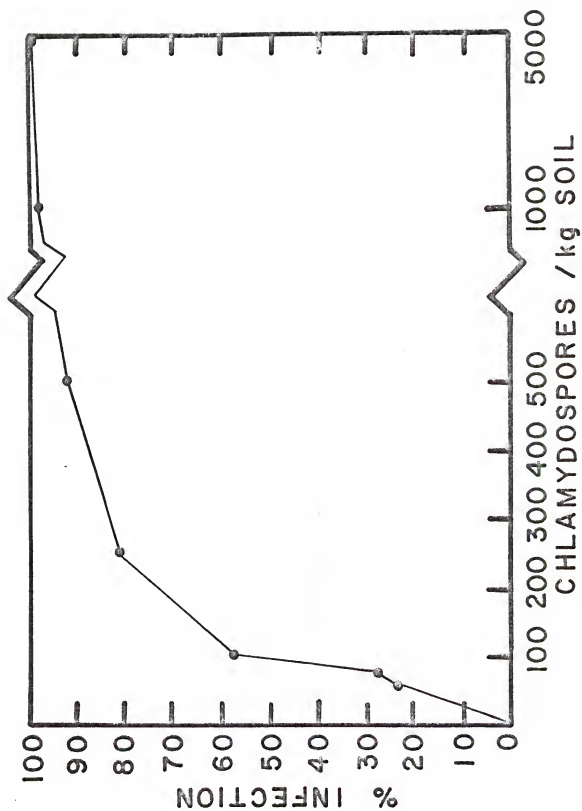


Fig. 2. The relationship of density of chlamydospores
(Log10) of Phytophthora parasitica var. nicotianae
in soil to percentage of infection $\text{Log}_{10} (\log_e 1/(1-x))$,
x = proportion of plants infected 50 dy after planting.

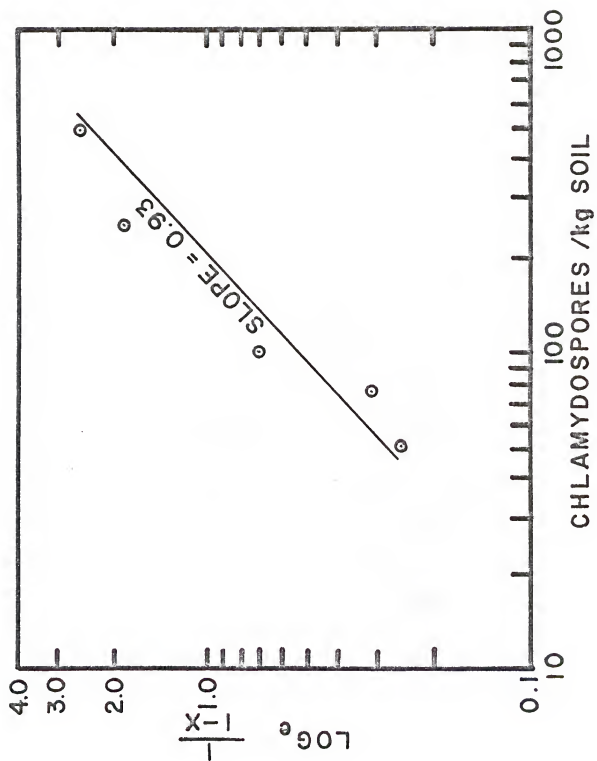


Fig. 3. The relationship of numbers of zoospores of Phytophthora parasitica var. nicotianae per tobacco plant to percentage infection 28 dy after inoculation.

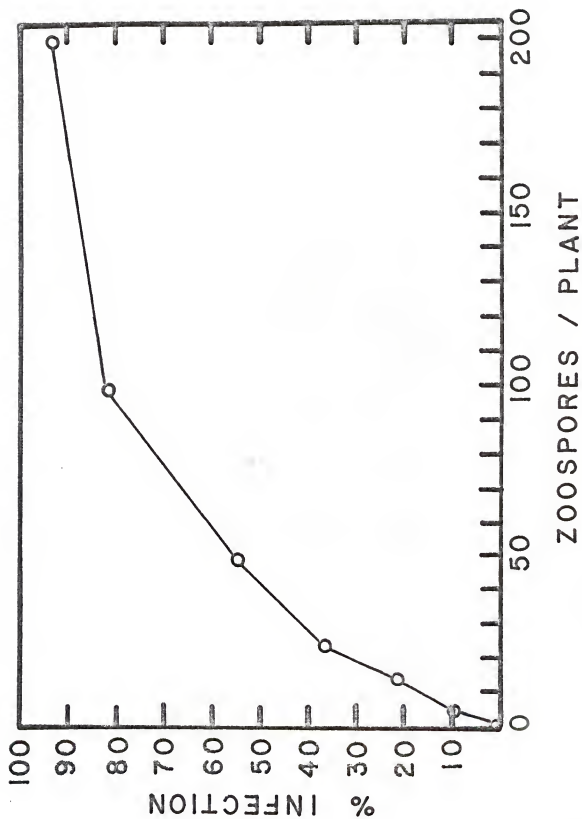
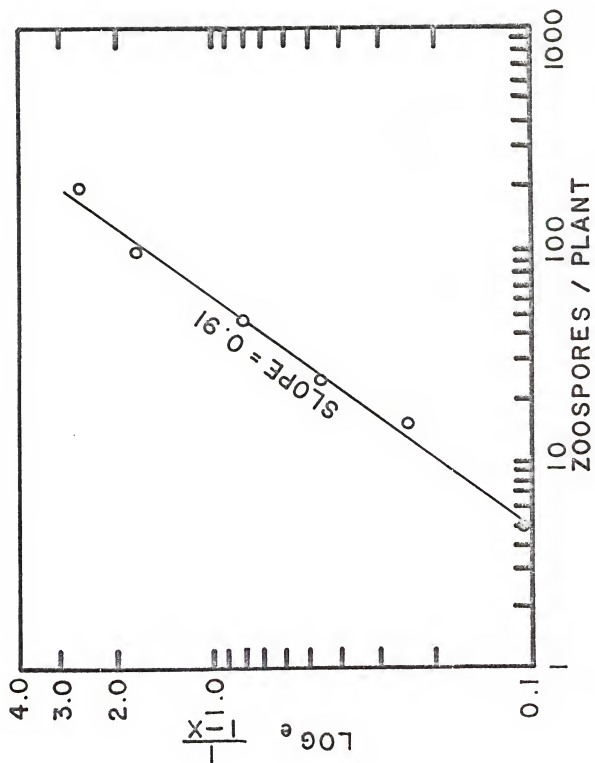


Fig. 4. The relationship of number of zoospores
(Log10) of Phytophthora parasitica var. nicotianae
per tobacco plant to percentage of infection
Log10 (Log_e $1/(1-x)$), x = proportion of plants
infected 28 dy after inoculation.



Discussion

Lower inoculum densities of P. parasitica var. nicotianae were required for 50% infection of tobacco plants in this study than have been required for the same level of infection of other hosts by various species of Phytophthora (31). Approximately 132 chlamydospores per kilogram of soil were required for 50% infection and mortality of young, susceptible tobacco plants grown for 50 dy in individual containers under growth-room conditions. Mitchell (31) reported that 50% infection of milkweed vine by P. citrophthora and of papaya by P. palmivora required 600 to 900 chlamydospores per kilogram of soil, respectively. In his experiments plants were exposed to a smaller amount of infested soil (15 g per container) than was used in the tobacco experiments, and the length of exposure of the plants to the soil was 1-2 wk compared to 7 wk with tobacco. However, the total number of spores to which each plant was exposed that resulted in 50% infection was similar in the three systems. Only 9, 9, or 14 chlamydospores of P. parasitica var. nicotianae, P. citrophthora, or P. palmivora per plant were required

for 50% infection of tobacco, milkweed vine, and papaya, respectively.

Gooding and Lucas (13) reported that the percentages of mortality of tobacco plants increased with an increase in zoospore inoculum. They found that 10^4 zoospores per plant were required for approximately 50% mortality of susceptible tobacco plants and that 10^6 zoospores were required for 100% mortality of plants within 2 wk after zoospores were injected into soil around plants. Recently, however, low numbers of zoospores of Phytophthora spp. have been shown to be able to infect and cause death of host seedlings when applied directly onto the plant surface in water (28, 34). Pratt et al. (34) found that 30% of the cotyledons of susceptible alfalfa seedlings became infected after inoculation with one zoospore of P. megasperma per cotyledon. McIntyre and Taylor (28) sprayed a zoospore suspension of P. parasitica var. nicotianae on whole tobacco seedlings that resulted in approximately eight viable zoospores on or near each plant. This inoculum level resulted in sufficient disease to allow distinction between susceptible and resistant varieties based on the development of disease within 5 dy after inoculation. The experiments in this study were designed to provide conditions favorable for zoospore movement and presumably to be similar to conditions in the field where zoospores are important in secondary

spread of disease (i.e., where zoospores move in saturated soil or in surface water). Only 5, 42, and 158 zoospores of P. parasitica var. nicotianae per plant were required for 10, 50, and 90% infection of susceptible tobacco plants respectively. Mitchell (31) reported that 250-280 zoospores of P. cryptogea, Pythium aphanidermatum, and Pythium ostracodes were required for 50% infection of watercress, tomato, and cotton, respectively, under similar experimental conditions. Fewer zoospores of P. parasitica var. nicotianae may have been required for the infection of tobacco, as compared to infection in the above host-pathogen combinations, because the young tobacco plants were succulent and had lower leaves as well as stem tissue exposed to zoospore-infested water, whereas only stems were exposed in the other combinations.

The slopes of the log-log transformations of the relationships of both zoospores and chlamydospores to the proportion of infected plants approached 1.0, which indicates direct proportionality. Other studies which have employed chlamydospores as inoculum have resulted in similar slopes, but those using zoospore inoculum resulted in slopes close to 0.67 (31, 32). If slopes of less than 1.0 result from competition for susceptible sites at higher inoculum levels or from other factors that reduce survival or effectiveness of the various spore forms in infection (2, 31, 32, 49), the differences

in slopes obtained with zoospores and various hosts under similar conditions may be explained by the availability of susceptible host tissue for infection or a reduced effectiveness of zoospores.

The role of oospores of heterothallic species of Phytophthora in the development of disease is unknown. In this study none of the oospores of P. parasitica var. nicotianae were observed to germinate in vitro or to infect tobacco roots in soil. Similar procedures to those employed in this study have been used to obtain oospores of homothallic species of Phytophthora that resulted in plant infection [(3), and Baumer and Erwin, unpublished]. Banihashemi and Mitchell (3) obtained approximately 45 and 100% infection of safflower seedlings planted in soil infested with 100 or 50,000 oospores of P. cactorum per gram of soil, respectively. Baumer and Erwin (unpublished), however, found that inoculum densities of only 20 or more Gluculase-treated oospores of P. megasperma per kilogram of soil (0.02 oospores per gram of soil) resulted in 50% death of susceptible seedlings of alfalfa within 30 dy. Multiple plants per container were used in both of the above studies which probably resulted in secondary spread of the fungi; thus, it is difficult to ascertain the inoculum densities required for specific higher levels of primary infection. These studies do demonstrate the ability of oospores of these homothallic

Phytophthora spp., which do not produce chlamydospores and depend on oospores for long-term survival and subsequent primary infection, to germinate in soil and infect host roots. Although the results with oospores of P. parasitica var. nicotianae are discouraging because no infection occurred even when the plants were exposed to up to 500 oospores per gram of soil for up to 75 dy, additional studies still are needed to elucidate the role of oospores of heterothallic species of Phytophthora in disease cycles. Some of the important factors that should be examined include the age and source (various cultures or host tissue) of oospores, susceptibility of various host tissues to infection, and, most importantly, the physical, biological, and chemical factors in the soil that affect the formation, survival, and germination of oospores.

The techniques used in this study, along with the host-pathogen system of tobacco and P. parasitica var. nicotianae, supported models with either chlamydospores or zoospores that had approximately the same percentages of both infection and mortality at specific inoculum levels. Mitchell (30) observed that the same levels of rye infected by Pythium myriotylum subsequently died under favorable conditions; however, most studies on the relationship of inoculum densities of species of Phytophthora and Pythium to disease incidence either have not

quantified percentages of infection with individual plants, or have employed host-pathogen combinations that require much higher inoculum levels for mortality than for infection (31). The tobacco-*P. parasitica* var. *nico-tianae* model saves time and expense in epidemiological studies because individual plants will not have to be plated on selective media to differentiate critically between infection and death. It is also a good model to differentiate time or other factors required for infection as compared to death.

SECTION II
THE INFLUENCE OF A FUNGICIDE ON THE EPIDEMIOLOGY OF
BLACK SHANK OF TOBACCO

Introduction

Black shank of tobacco (Nicotiana tabacum L.), which is caused by Phytophthora parasitica Dast. var. nicotianae (Breda de Haan) Tucker (= P. nicotianae Breda de Haan var. nicotianae Waterhouse), is a serious disease in most of the tobacco growing areas of the United States (26). The incidence of black shank increases with increasing levels of inoculum (11, 13, 19, 26). Although factors that affect the epidemiology of black shank, such as pH, temperature, and water relations, have been investigated, little has been reported on the quantitative relationship of inoculum to the development of disease over time (26). Flowers and Hendrix (11) were unable to determine reliably the populations of P. parasitica var. nicotianae in fields at the time of planting and did not relate initial populations to specific amounts of disease development during the growing season; they showed, however, that populations of the fungus increase in the rhizospheres of host plants. They suggested that

differences in the rate of black shank development could be due to differences in population densities at the beginning of the season. Plants of Burley 21, a cultivar of tobacco susceptible to P. parasitica var. nicotianae, were killed 3-4 wk after transplants were placed in a field where the inoculum density was less than 10 propagules per gram of soil (11), and after 2 wk in pots of naturally infested soil containing an initial population of 34 propagules per gram of soil (12). Control of black shank has been inadequate with available chemicals, and even resistant cultivars often become diseased after exposure to high levels of inoculum. Recently, a group of acylalanine derivatives which systemically control various diseases caused by fungi in the oomycetes have become available from CIBA-GEIGY Corp. for experimentation (5, 38, 40, 47). Diseases caused by species of Phytophthora, Pythium, Bremia, Plasmopora, Pseudoperonospora, and Sclerospora are among those controlled by these compounds. One acylalanine analogue was inhibitory to P. parasitica var. nicotianae in vitro and gave good control of black shank of tobacco in preliminary field tests in 1976 (33). This compound, which has the systematic chemical name of N-(2,6-dimethylphenyl)-N-(methoxyacetyl)-alanine methyl ester, was tested as CGA 48988 (=GA-1-82) and given the trade name Ridomil in Europe (40, 47).

Most evaluations of black shank are based on yield losses, disease indices, or the percentages of plants dead at the end of the season. Other quantitative parameters such as the times required for disease to reach specific severities and apparent infection rates (48) allow more accurate interpretation of the influence of a fungicide on disease progression. The objective of this study was to determine the influence of CGA 48988 on the relationship of inoculum density to the rate and severity of black shank development in susceptible and moderately resistant cultivars of tobacco under plant growth room, greenhouse, and field conditions.

Materials and Methods

The isolate of P. parasitica var. nicotianae (P-230) from tobacco used in this study was obtained from the collection of Phytophthora spp. maintained at the Department of Plant Pathology, University of California, Riverside. It was maintained on V-8 Juice agar and transferred monthly.

Chlamydospores produced by the method of Tsao (45) were used as inoculum in the growth room and greenhouse studies. Known numbers of chlamydospores, free of hyphal fragments and other spores, were obtained with the method described by Ramirez and Mitchell (35).

Growth room experiments were conducted using raw or autoclaved (2 hr on two successive days) Astatula sand (pH of 6.2 in a 1:2 suspension of soil in 0.01 M CaCl_2). The infested-soil-layer technique used by Mitchell (30) was used to allow noninjured plant roots to grow into infested soil. Soil was infested with known numbers of chlamydospores to establish various inoculum densities, mixed thoroughly, and adjusted to a final water content of 5% (w/w). Sixty-five grams of soil infested with 0, 0.5, 5, or 50 chlamydospores per gram of soil were layered over 15 g of autoclaved builder's sand in 100-ml polypropylene beakers which had small holes at the base to provide drainage. The infested layer was covered with

35 g of noninfested soil into which a 1-mo-old tobacco seedling of the susceptible cultivar Hicks was transplanted. The soil surface was covered with autoclaved vermiculite, and the beakers were placed on wire screen frames to prevent water movement among beakers. The seedlings had been grown for 1 mo in autoclaved vermiculite and fertilized weekly with 50 ml of a nutrient solution (15) diluted to half strength. The fungicide was incorporated at 0, 1, 5, or 25 mg active ingredient (ai) of a 25% WP of CGA 48988 per kilogram of air-dried soil (ppm) into both the infested and noninfested soil at each inoculum density. Experiments included 15 replicates of each treatment and were repeated three times. Plants were maintained in a growth room for 50 dy at 25-27 C and 12 hr of light (4,000 lx at the level of the plants). Plants were watered from the top with 15 ml of tap water per plant daily and 15 ml of half-strength nutrient solution twice a week.

Disease development, as related to inoculum density and fungicide treatment, also was evaluated under greenhouse conditions in 10-cm-diameter pots. Astatula sand, treated with 1 kg of methyl bromide per 200 kg of soil, was infested with 0, 0.5, 5, or 50 chlamydospores per gram of soil. Two hundred grams of infested soil were placed in each pot and covered with 200 g of noninfested soil into which a 6- to 8-wk-old Hicks

seedling was transplanted. Tobacco transplants had been³³ grown in methyl bromide-treated soil in 10-cm-square trays and fertilized twice a week with 50 ml of the nutrient solution per tray. The fungicide was incorporated at 0, 1, 10, and 50 ppm into both the infested and noninfested soil layers in the pots. Plants were watered daily with 50 ml of water and twice a week with 50 ml of the nutrient solution per plant. Temperatures in the greenhouse fluctuated from 19 to 32 C. Treatments consisted of 15 plants each, and the experiments were conducted three times.

Plants in beakers and in pots were inspected daily for wilting and discoloration of the stems. Dying plants were harvested 50 or 100 dy after transplanting from beakers or pots, respectively. Plant roots were washed, dipped in 70% ethyl alcohol, rinsed in sterile distilled water, blotted dry, and plated on the PVP selective medium of Tsao and Ocaña (46). The plates were observed for growth of P. parasitica var. nicotianae from the roots after incubation for 48 hr in the dark at 25 C.

The development of black shank was observed in a field near Quincy, FL, that had been artificially infested with P. parasitica var. nicotianae 7 yr prior to this study and then planted with tobacco in subsequent years. Split-plot experiments with a randomized complete block design of three replicates were used. Fungicide

treatments were whole plots of four rows each, and plant cultivars were subplots. Each subplot consisted of two rows (15 plants per row) of the susceptible tobacco cultivar, Hicks, or two rows of the moderately resistant cultivar, Speight G-28. Plants were watered with an overhead irrigation system, and cultural practices were similar to those used by commercial growers in the area. Nine-wk-old tobacco plants from fumigated plant beds were transplanted into the field.

Approximately 130 ml of a transplant solution that contained 150 ppm ai of 25% CGA 48988 WP were added to the hole in which each plant was set in the field. Plants were inspected weekly for symptoms of black shank and considered to be dead when they had blackened stems and had wilted permanently. Soil samples from the top 10 cm of the soil were taken from the rhizospheres of 12 plants in each subplot and combined. The position of each plant was marked to permit sampling after plant death. Twenty grams of each combined sample were diluted 1:10 in 0.25% water agar and 1-ml aliquots were spread over the surface of 10 agar plates of a selective medium containing: 10 mg pimarcin (Delvocid, 50% ai, Gist-Brocades N.V., Delft, Holland), 250 mg ampicillin (Polycillin-N, 81% ai, Bristol-Meyers Co., Syracuse, NY 13201), 10 mg rifampicin (Rifamycin SV, 100% ai, Sigma

Chemical Co., St. Louis, MO 63178), 100 mg pentachloro-nitobenzene (Terraclor, 75% ai, Olin Mathieson Chemical Corp., Little Rock, AR 72203), 50 mg 3-hydroxy-5-methylisoxazole (Tachigaren, 99%, Sankyo Co., Yasu, Tokyo, Japan), and 17 g Difco cornmeal agar in 1.0 liter de-ionized water. After incubation for 48 hr at 25 C in the dark, the soil suspension was washed from the agar surface under a slow stream of tap water, and fungal colonies were counted.

In the growth room, greenhouse, or field, an inoculum density at planting of approximately 0.5 propagule of P. parasitica var. nicotianae per gram of soil resulted in 93 to 100% mortality of Hicks plants and apparent infection rates (r) of 0.08-0.11/unit/dy (Table 1). Apparent infection rates were determined by linear regression analyses of time in days versus percentages of mortality (dead or moribund plants) corrected for multiple infections. $\log_e 1/(1-x)$, where x equaled the proportion of dead plants, was used for the growth room and greenhouse data because individual plants were exposed to defined initial inoculum in individual pots. $\log_e x/(1-x)$ was used for field data because secondary infection could have resulted from the spread of inoculum in the field. When dead plants were plated on PVP, P. parasitica var. nicotianae grew from the roots and blackened stems of all plants counted as dead. Although raw soil was not used routinely in this study, the 87% mortality and $r=0.08/\text{unit}/\text{dy}$ at 0.5 chlamydo-spores per gram of raw soil in beakers or pots obtained in two tests were similar to values obtained at 0.5 chlamydo-spores per gram of soil under the same conditions in autoclaved or methyl bromide-treated soil.

When inoculum densities were increased from 0.5 to 5 and 50 chlamydospores per gram of soil, r was progressively greater in the growth room but not in the greenhouse (Table 1). Mortality occurred soonest in both the growth room and greenhouse at the higher inoculum densities. The times required for 10% of the plants to die (t_{10}) (as determined by linear regression analyses) were 23, 20, and 17 dy at 0.5, 5, and 50 chlamydospores per gram of soil, respectively, in the growth room; the corresponding values at these propagule densities in the greenhouse were 35, 24, and 21 dy. Although symptoms occurred sooner in the growth room than in the greenhouse, the times required for plant death to increase from 10 to 50% (t_{10-50}) at each inoculum density were similar in the two environments. The number of days required for mortality to increase from 10 to 90% (t_{10-90}) was about the same at 0.5 chlamydospores per gram of soil in both the growth room and the greenhouse, but t_{10-50} was shorter at 5 and 50 chlamydospores per gram of soil in the growth room than in the greenhouse. The t_{10-90} was more rapid at 50 than at 5 chlamydospores per gram of soil in the growth room, but not in the greenhouse.

Although black shank occurred sooner in the field ($t_{10}=18$ and 6 dy in 1976 and 1977, respectively) than in the greenhouse ($t_{10}=35$ dy) or in the growth room ($t_{10}=23$ dy), t_{10-50} and t_{10-90} were longer in the field

than under artificial conditions (Table 1). The t_{10-50} (24 and 22 dy in 1976 and 1977, respectively) and t_{10-90} (49 and 44 dy in 1976 and 1977, respectively) in the field experiments were similar for each of the two years.

No plants were killed under growth-room conditions at 0.5 chlamydospore per gram of soil when 1 or 5 ppm of CGA 48988 was incorporated into the soil at the time of planting (Table 1). Percentages of mortality in pots in the greenhouse were 93, 60, 7, and 0% at 0.5 chlamydospore per gram of soil with 0, 1, 10, and 50 ppm of the fungicide, respectively. No infection occurred in plants grown in pots containing 50 ppm of CGA 48988 after 100 dy at inoculum levels up to 50 chlamydospores per gram of soil. Phytotoxicity due to the fungicide was not evident at the concentrations that controlled black shank in greenhouse or growth-room studies. Root weights were not reduced in the absence of the fungus at fungicide concentrations up to 10 ppm in the growth-room experiments, and at 0 or 0.5 chlamydospore per gram of soil there were no differences in root weights with 0, 5, or 25 ppm of the fungicide incorporated into autoclaved or raw soil. In the field, however, plants treated with the fungicide appeared to be stunted at the beginning of the season, but no differences between the treated and nontreated plants were evident at the end of the season.

An arithmetic plot of percent mortality of nontreated susceptible plants in the field versus time results in an s-shaped disease progress curve; disease development was most rapid between 21 and 42 dy after planting (Fig. 5). The development of black shank was delayed by the fungicide treatment until 56 dy after planting, but then the disease progressed more rapidly than in the control. Black shank developed more slowly in the resistant plants than in the susceptible plants; at the end of the season 61% of the nontreated resistant plants and only 10% of the fungicide-treated resistant plants were dead. Although 100% of the treated and nontreated susceptible plants were dead at the end of the season (94 dy after transplanting), the delay in disease development in treated plants resulted in a dry-leaf yield from six leaf harvests of 1,964 kg/ha, which was significantly ($P=0.05$) greater than the yield of 246 kg/ha in the control. Yield of resistant plants also was increased significantly ($P=0.05$) from 2,882 kg/ha for nontreated plants to 4,102 kg/ha for plants treated with the fungicide.

The apparent infection rates were lower for resistant plants in the field than for susceptible plants (Fig. 6). Excellent protection from black shank was provided by treating the resistant cultivar Speight G-28 with CGA 48988. As indicated above, disease was delayed

in susceptible plants treated with the fungicide, but after 56 dy it progressed faster ($r=0.22/\text{unit}/\text{dy}$) than in the nontreated susceptible plants ($r=0.10/\text{unit}/\text{dy}$). The apparent infection rate for the fungicide-treated susceptible plants was calculated from disease observations made from 49 to 70 dy after planting.

The population of P. parasitica var. nicotianae in the field at the time of planting was 0.75 propagule per gram of soil, but populations increased to a maximum of over 250 propagules per gram of soil in samples from the rhizospheres of nontreated, susceptible plants between 13 and 46 dy after planting (Fig. 7). Maximum populations of the fungus in the rhizospheres of fungicide-treated, susceptible plants and nontreated, resistant plants were 145 and 112 propagules per gram of soil, respectively, but they were not attained until approximately 65 dy after planting. Populations of P. parasitica var. nicotianae did not reach the maximum of 105 propagules per gram of soil in the rhizosphere of resistant plants treated with the fungicide until after the time of final leaf harvest. After the death of infected plants, populations decreased in all treatments. Soil samples collected from the rhizospheres of Hicks plants in the field in 1976 were plated on the gallic acid medium developed by Flowers and Hendrix (10), but very low levels of

P. parasitica var. nicotianae were detected. The maximum rhizosphere population of 32 propagules per gram of soil was detected 70 dy after planting.

TABLE 1. The influence of inoculum density of *Phytophthora parasitica* var. *nicotianae* and the fungicide CGA 48988^a on the development of black shank in the susceptible tobacco cultivar Hicks under growth room, greenhouse and field conditions.

Conditions ^b	Propagules ^c /g of soil (no.)	CGA 48988 (ppm)	Disease development ^d			Mortality at Harvest ^e (%)	r ^f /unit/day
			t ₁₀ (days)	t ₅₀ (days)	t ₉₀ (days)		
Growth room	0.5	0	23	7	24	93	.09
	0.5	1	—	—	—	0	0
	0.5	5	—	—	—	0	0
	50.0	0	25	4	15	100	.14
	50.0	0	17	4	13	100	.17
Greenhouse	0.5	0	35	7	25	93	.09
	0.5	1	36	30	110	60	.02
	0.5	10	—	—	—	7	0
	0.5	50	—	—	—	0	.5
	50.0	0	24	5	15	100	.10
Field 1976	50.0	0	21	5	13	100	.10
	50.0	50	—	—	—	0	0
	Nil ^g	0	15	15	45	100	.02
Field 1977	0.75	0	6	22	44	100	.10
	0.75	150	53	10	20	100	.22 ^h

^a25% wettable powder of N-(2,6-dimethylphenyl)-N-(ethoxyacetyl)-alanine methyl ester (CGA 48988 = CA-1-E2, CIFA-GEI-Y, Corp.) was incorporated into soil (w/w) for greenhouse and growth room tests and was added as 130 ml of a 150 ppm solution to the soil around each transplant at the time of planting in the field.

^bGrowth rooms provided 12 hr of light (4,000 lx at the level of the plants) at 25°C; temperatures varied from 19-32°C in the greenhouse.

^cInoculum for growth room and greenhouse tests was established as chlamydospores/g of soil; populations in field soil were based on dilution plate assays and converted to propagules per gram of soil.

^dDays (interpolated from linear regression analyses) required for 10% plant death (t₁₀) or for mortality to increase from 10 to 50% (t₅₀) and from 10 to 90% (t₉₀).

^ePlant mortality with 15 plants per treatment 50, 100, and 94 dy after planting in the growth room, greenhouse, or field, respectively.

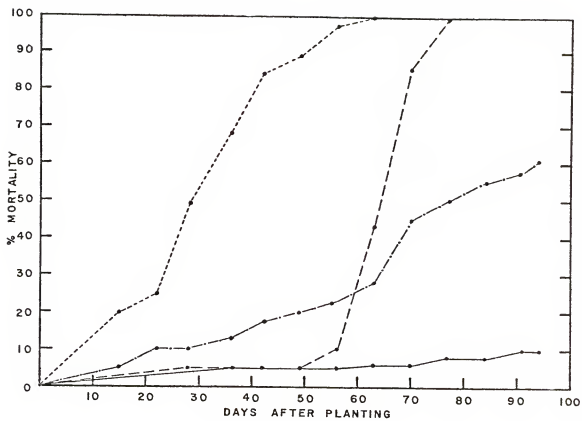
^fApparent infection rates, r, were slopes of linear regression lines of Log_e 1/(1-x) on time in the growth room and greenhouse and Log_e x/(1-x) in the field; x = 100 X percent death due to black shank.

^g— = insufficient disease for calculation.

^h*Phytophthora parasitica* var. *nicotianae* was not detected before planting.

ⁱCalculation for r for the fungicide-treated plants in 1977 was based on disease incidence from 49 to 70 days after planting.

Fig. 5. Rate of plant death in the field for tobacco plants of the susceptible cultivar Hicks (----), CGA 48988-treated Hicks (— — —), resistant cultivar Speight G-28 (---), and CGA 48988-treated Speight G-28 (—●—●). Percentage of mortality and days after planting.



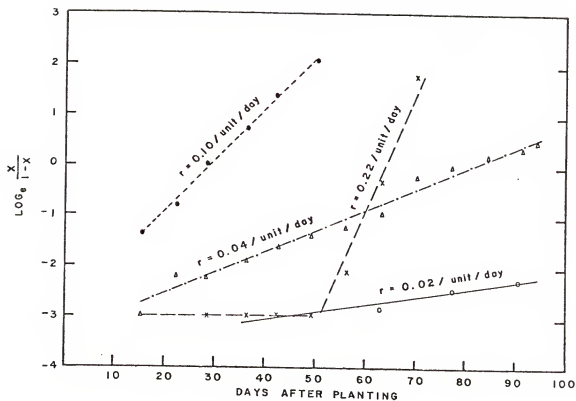


Fig. 6. Rate of plant death in the field for tobacco plants of the susceptible cultivar Hicks (---), CGA 48988-treated Hicks (x---x), resistant cultivar Speight G-28 (Δ---Δ), and CGA 48988-treated Speight G-28 (o---o). Regression lines for mortality (x = proportion of plants dead) and days after planting.

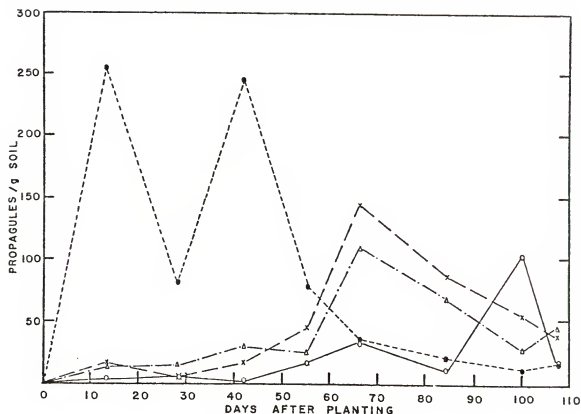


Fig. 7. Populations of *Phytophthora parasitica* var. *nicotianae* in the rhizospheres of tobacco plants of the susceptible cultivar Hicks (---•---), CGA 48988-treated Hicks (x---x), resistant cultivar Speight G-28 (-.-Δ-), and CGA 48988-treated Speight G-28 (○—○).

Discussion

This study confirms that severe black shank can occur in soil with very low initial inoculum levels of P. parasitica var. nicotianae. All of the susceptible Hicks and 58-61% of the moderately resistant Speight G-28 plants died in three separate fields in north Florida which had initial populations of P. parasitica var. nicotianae of 0.2, 0.4, and 0.75 propagule per gram of soil (Mitchell, unpublished). Flowers and Hendrix (11, 12) also reported severe disease under greenhouse or field conditions where initial populations of P. parasitica var. nicotianae were low. Kannwischer and Mitchell (unpublished) found that approximately 0.2 chlamyospore per gram of soil was required for 50% infection of tobacco seedlings by P. parasitica var. nicotianae under growth room conditions. Studies with other Phytophthora spp. indicate that 0.1 to 1 chlamyospore per gram of soil can cause 50% infection of papaya or milkweed vine (31). In this study initial inoculum densities of 0.5 chlamyospores per gram of soil resulted in mortality and apparent infection rates under growth room and greenhouse conditions comparable to those in the field at approximately the same initial inoculum densities.

The similarity in the values obtained under controlled and field conditions may be due in part to the

use of inoculum in the growth room and the greenhouse in the form of chlamydospores, which most closely represent the type of survival or resting structures that are present in the soil or plant debris in the field at the time of planting. The use of autoclaved or methyl bromide-treated soil in these tests is considered reliable because results of experiments using raw, methyl bromide-treated, or autoclaved soil, did not differ greatly. Earlier initiation of disease and higher r at high inoculum densities in the growth room than in the greenhouse may be due to more favorable environmental conditions for disease development in the growth room or to greater susceptibility of the younger seedlings in the growth room than in the greenhouse experiments. The times required for mortality and r in the field were more closely simulated in the greenhouse than in the growth room.

Model systems in the growth room and greenhouse make possible the evaluation of a large number of plants in a relatively small area under controlled environmental conditions. Systems such as those used in this study can be useful in the quantitative evaluation of factors affecting disease, such as environmental conditions, host resistance or tolerance, and biological or chemical control.

In this study, CGA 48988 was efficacious in the control of black shank at very low concentrations in the growth room and greenhouse. The fungicide at 1 ppm gave complete control of black shank in seedlings grown in

small volumes of artificially infested soil in the growth room for 50 dy. When plants were grown in larger volumes of infested soil in pots in the greenhouse for 100 dy, only 7% of the plants were killed at 10 ppm of CGA 48988; even at an initial inoculum density of 50 chlamydo-spores per gram of soil, none of the plants was killed after 100 dy in pots and P. parasitica var. nicotianae was not isolated from the roots of plants exposed to the fungicide at 50 ppm. Phytophthora parasitica var. nicotianae was not completely inhibited at similar levels in vitro; although P. cryptogea, P. palmivora, and P. parasitica were inhibited almost completely at 5 ppm of CGA 48988 in V-8 Juice agar, five isolates of P. parasitica var. nicotianae were inhibited only 60% at 5 to 50 ppm (Mitchell, unpublished). Kelley (22) observed that radial growth of four other Phytophthora spp. was reduced in V-8 Juice agar from 25-70% by 1 ppm of CGA 48988. Under field conditions 150 ppm of the fungicide delayed disease in susceptible plants for approximately 50 dy, but then disease progressed rapidly. Good control was obtained when moderately resistant plants were treated with CGA 48988. Increases in rhizosphere populations of P. parasitica var. nicotianae corresponded to the time of rapid disease development in each treatment. Flowers and Hendrix (11, 12) also concluded that increases in rhizosphere populations were associated with pathogenesis.

The quantitative evaluation of disease over time should prove useful in recommendations for the application of control measures. The lack of disease control in susceptible plants beginning about 50 dy after planting was due possibly to a reduction in efficacy of the fungicide over time, to changes in the physiology of the plant, or to the movement of the roots into infested soil outside of the treated area and subsequent infection. It is of interest that both the first leaf harvest and flowering occurred about 50 dy after transplant. Further considerations for the use of CGA 48988 to control black shank should include the application of a second fungicide treatment about 40-45 dy after planting or the use of a broadcast treatment to increase the volume of treated soil.

The logit transformation $\text{Log}_e x/(1-x)$ was used to correct for multiple infections in the field because black shank was considered to be a multiple cycle ["compound interest" (48)] disease, and because this transformation allowed interpolations for t_{10} , t_{10-50} , and t_{10-90} that corresponded closely to those that actually occurred. Apparent infection rates have not been determined for other multiple cycle, plant diseases caused by soilborne pathogens and the treatment of data in this study for the determination of r should be

viewed as an initial attempt to apply a known transformation to such a disease. Additional work will be required to determine which transformation(s) will best express data for the epidemiological characterization of multiple cycle, soilborne diseases. Since the significance of secondary dissemination of inoculum by movement of infested soil or water has not been determined critically, it will be particularly important to evaluate disease gradients from point sources of inoculum.

SECTION III
FACTORS AFFECTING THE SURVIVAL OF PHYTOPHTHORA
PARASITICA VAR. NICOTIANAE IN SOIL

Introduction

The occurrence of black shank of tobacco (Nicotiana tabacum L.), caused by Phytophthora parasitica Dast. var. nicotianae (Breda de Haan) Tucker [= P. nicotianae Breda de Haan var. nicotianae Waterhouse], has been reported after various crop rotation sequences in which more than 4 yr passed between tobacco crops (1). Various factors such as soil moisture, temperature, pH, and biotic factors are known to affect the survival of P. parasitica var. nicotianae in soil (8). Flowers and Hendrix (12) reported that the presence of different host and nonhost roots and repeated freezing and thawing of the soil did not affect the survival of artificially established or natural populations of the fungus in soil maintained in pots for 3 mo. In a separate study, populations of P. parasitica var. nicotianae were sampled in two fields from the root zones of tobacco plants which were susceptible, moderately resistant, or resistant to the

fungus (11). They were unable to detect reliably the initial population of P. parasitica var. nicotianae prior to planting time, but they found that population increases corresponded to pathogenesis and mortality of plants. When soil from the region of the roots of the plants was sampled in the winter and spring following the growing season, declines in the populations of the fungus were observed. Because their samples from the field and from pots were limited to the soil around plants, they were unable to determine whether declines in the number of propagules detected were due to the depletion of infested soil by repeated sampling or if the reduction in population was a phenomenon that occurred throughout the soil (11).

The population dynamics of P. parasitica var. nicotianae in soil maintained under controlled environmental conditions have not been evaluated critically. Such evaluations, particularly when compared to field situations, will provide information on the survival and pathogenic capabilities of inoculum of P. parasitica var. nicotianae in soil that will be required for forecasting this disease in the field.

In this study artificially and naturally established populations of P. parasitica var. nicotianae in different soils maintained at two temperatures and under three moisture regimes were monitored using a selective plating

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technique. The abilities of populations of the fungus to cause disease were determined using a plant bioassay technique. Populations of P. parasitica var. nicotianae also were monitored in two fields by sampling from the former root zones of individual, dead, tobacco plants of susceptible and moderately resistant cultivars, or by sampling soil from portions of the fields that had been disked and left fallow after a growing season in which severe black shank had occurred in the fields.

Materials and Methods

Laboratory studies were conducted to evaluate the survival of isolate P-230 of P. parasitica var. nicotianae (obtained from the collection of Phytophthora spp. at the University of California, Riverside) in artificially infested raw Astatula sand, autoclaved Astatula sand (2 hr on two successive days), and autoclaved Ruston loamy-fine sand. Survival of this fungus as a resident population (approximately 13 propagules of P. parasitica var. nicotianae per gram of soil) also was evaluated in Ruston loamy-fine sand collected from a field that was infested artificially with P-230 in 1970 and was planted in tobacco in subsequent years. Soils were maintained at 10 C or 25 C under three moisture regimes.

The saturation percentages [$SP = 100 \times (\text{total weight of water at saturation}) / (\text{weight of oven dry soil})$] were determined by a soil-paste method (37). The moisture regimes included a wet regime (maintained at 27% saturation), a dry regime (maintained at 6.8% saturation), and a wetting-drying regime (repeatedly adjusted to 27% saturation and allowed to dry to 6.8% saturation). The saturation percentages were 30.0, 29.7, 36.9, and 39.8 for autoclaved Astatula sand, raw Astatula sand, autoclaved Ruston loamy-fine sand, and raw Ruston loamy-fine sand,

respectively. The percent moisture content (P_w) of each soil was determined on a dry weight basis (37) (Table 2).

Six 500 g lots each of autoclaved Astatula sand, raw Astatula sand, and autoclaved Ruston loamy-fine sand were infested with 250 chlamydo spores per gram of wet soil as described previously (20). Four lots of each soil and four lots of raw Ruston loamy-fine sand with resident populations of the fungus were adjusted with deionized water to a final moisture content of 27% saturation, and the two remaining lots of each soil (including two lots of raw Ruston loamy-fine sand) were adjusted to a final moisture content of 6.8% saturation. All of the lots except two of each soil at 27% were placed in covered 1-liter plastic containers that had two small holes near the top to allow air exchange with minimal loss of water due to evaporation. The remaining soil lots at 27% saturation were placed in containers with 10 holes near the top of each to facilitate drying of the soil for the wetting-drying regime. Lots of soil at each moisture regime were placed in incubators at 10 or 25 C.

Twenty-four 1-kg lots each of autoclaved Astatula sand, raw Astatula sand, and autoclaved Ruston loamy-fine sand were infested with 5 chlamydo spores per gram of wet soil. All lots of each soil and 24 1-kg lots of

TABLE 2. Soil saturation percentages and the corresponding soil moisture percentages.

Soil type		Soil moisture percentages ^a at saturation percentages ^b	
		6.8	27.0
Ruston loamy- fine sand	autoclaved	2.5	10.0
	raw	2.7	10.8
Astatula sand	autoclaved	2.1	8.1
	raw	2.0	8.0

^aSaturation percentage = $100 \times (\text{grams of water at saturation}) / (\text{grams of oven dry soil})$ as determined by a soil paste method.

^bSoil moisture percentage = $100 \times (\text{grams of water}) / (\text{grams of oven dry soil})$.

^cSoil was autoclaved 2 hr on two successive days.

raw Ruston loamy-fine sand containing resident populations of the fungus were adjusted with deionized water to a moisture content of 27% saturation. One-half of the lots of each soil were placed in containers with two holes (wet regime) and the other half in containers with 10 holes (wetting-drying regime). The containers were placed in an incubator at 25 C in the dark.

All of the soil lots were weighed weekly and the moisture of the soil in a wet or a dry regime was adjusted with deionized water to the original moisture content. Soil lots maintained under a cyclic wetting-drying regime were allowed to dry to a moisture content of 6.8% saturation before the moisture was adjusted back to a moisture content of 27% saturation.

The populations of P. parasitica var. nicotianae in each soil treatment were sampled 0, 1, 4, 7, 14, 30, 60, and 90 dy after initiation of the experiment. A sample of soil equivalent in dry soil weight to a 20 g sample of the same soil at the initial adjusted soil saturation percentage was diluted either 1:10 or 1:20 (depending on the initial infestation level or the results of the previous sampling) in 0.25% water agar. A 1-ml sample of the soil suspension was spread over the surface of each of 10 agar plates of a selective medium (20, 27), which consisted of 10 mg pimaricin (Delvocid, 50% ai,

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Gist-Brocades N. V., Delft, Holland), 250 mg ampicillin (Polycillin-N, 81% ai, Bristol-Meyers Co., Syracuse, NY 13201), 10 mg rifampicin (Rifamycin SV, 100% ai, Sigma Chemical Co., St. Louis, MO 63178), 100mg pentachloro-nitrobenzene (Terraclor, 75% ai, Olin Mathieson Chemical Corp., Little Rock, AR 72203), 50 mg 3-hydroxy-5-methylisoxazole (Tachigaren, 99%, Sankyo Co., Yasu, Tokyo, Japan), and 17 g Difco cornmeal agar in 1.0 liter of deionized water. After incubation for 48 hr at 25 C in the dark, the soil was washed from the surface of the agar and the colonies of P. parasitica var. nicotianae were counted. The ability of the fungus to infect tobacco plants was evaluated by previously described methods (20) 0, 1, 4, and 7 dy after raw Astatula sand, autoclaved Astatula sand, and autoclaved Ruston loamy-fine sand had been infested with 5 chlamydospores per gram of soil and maintained subsequently at 25 C under wet, and wetting-drying moisture regimes. The same procedures were used with raw Ruston loamy-fine sand with resident populations of the fungus. Sixty-five grams of infested soil were layered over 15 g of autoclaved builder's sand in each of fifteen 100-ml polypropylene beakers per treatment. The infested soil in each beaker was covered with 35 g of noninfested, autoclaved soil into which a 1-mo-old tobacco seedling

of the susceptible cultivar Hicks was transplanted. The soil surface was covered with autoclaved vermiculite to prevent rapid drying of the surface soil. Plants were placed in a plant growth room at 25 C with 12 hr of light (4,000 lx at the level of the plants), watered daily, and fertilized once a week with half-strength Hoagland's solution (15). The plants were observed daily for symptoms of black shank and considered dead when they had wilted permanently and had blackened stems. After 50 dy the roots and stems of surviving plants were washed in tap water, dipped in 70% ethyl alcohol, rinsed twice with deionized water, blotted dry, and plated on the selective medium. The plates were observed for growth of P. parasitica var. nicotianae from roots and stems of infected plants after 48 hr of incubation at 25 C in the dark.

Populations of P. parasitica var. nicotianae in soil were followed from August 1977 to June or September 1978 in tobacco fields in Gainesville and Quincy, FL, respectively. Both fields had been infested with P. parasitica var. nicotianae approximately 7 yr prior to this study and during the 1977 growing season 100% of the susceptible plants and 61-63% of the moderately resistant Speight G-28 plants were killed in both fields. The positions of black shank infected plants were marked

to allow samples of soil to be taken within a radius of 10-15 cm from the plant axis. Samples were also taken at random in the fields in areas that were disked in late September 1977 and in April 1978, and left fallow for the remainder of the sampling periods. Each soil sample consisted of one soil core (approximately 10 cm long with a 2.5-cm diameter).

One sample was taken near each of six plants of a susceptible cultivar, Hicks, and each of six plants of a moderately resistant cultivar, Speight G-28 in each of two plots in the field near Quincy, FL. The populations were monitored in six areas of the field and in two separate plots that consisted of four plant rows each (0.6 by 9 m each). The plots, designated C and D, were disked at the same time as the whole field. In each area 12 soil cores were collected randomly and bulked into one sample. Plants also were marked in a field in Gainesville, FL and samples were taken from the root zones of six Speight G-28, six Speight G-23 (a moderately resistant cultivar), and three Hicks plants. Six bulk samples of 12 soil cores each were taken randomly from disked areas of the field.

All soil samples were maintained in open plastic bags at room temperature until they were plated on the selective medium. The samples were plated within 24 hr

after collection. Twenty grams of each sample were diluted 1:10 and plated on the selective medium. The plates were maintained at 25 C in the dark and after 48-72 hr the soil was washed from the surface of the agar and the colonies of P. parasitica var. nicotianae were counted.

Results

When samples of autoclaved Ruston loamy-fine sand or Astatula sand maintained at 25 C were plated on the selective medium immediately after infestation with 250 chlamydospores of P.parasitica var. nicotianae per gram of soil, fewer propagules were detected than expected based on the number of propagules incorporated into the soil (Fig. 8, 9). The recovery of the fungus was greatest initially from both soils in the dry regime (6.8% saturation). The number of propagules of the fungus detected in autoclaved Ruston loamy-fine sand or autoclaved Astatula sand originally in the wet (27% saturation) or dry regimes increased when these soils were sampled after infested soil was maintained at 25 C for 1 dy. The number of propagules increased in the autoclaved Ruston loamy-fine sand in the wet regime at 25 C to a maximum of 456 propagules per gram of soil 14 dy after infestation. The maximum population in the dry regime (308 propagules per gram of soil) also was detected 14 dy after infestation, but the maxima of 273 propagules per gram of autoclaved Astatula sand in the wet and the wetting-drying soil moisture regimes were reached 1 dy after infestation. Populations declined in both soil types in all moisture regimes between 14 and 90 dy after infestation of the soil.

A reduction in recovery of propagules of P. parasitica var. nicotianae also was observed in the initial sampling of autoclaved Ruston loamy-fine sand and autoclaved Astatula sand maintained at 10 C (Fig. 10, 11). Populations of the fungus did not decline as rapidly at 10 C as at 25 C, and between 100 and 200 propagules of the fungus per gram of soil were detected after 90 dy at 10 C in all of the moisture regimes in both soils, except in the wet regime in autoclaved Astatula sand which fell to 40 propagules per gram of soil.

Propagules of the fungus in soil maintained at 25 C increased from 0 and 4 propagules per gram of autoclaved Astatula sand and Ruston loamy-fine sand initially in the wet regime sampled immediately following infestation with 5 chlamydospores per gram of soil, to 14 and 9 propagules per gram of soil, respectively, 1 dy after infestation (Fig. 12). Populations remained high for 60 dy and then decreased to near initial levels 90 dy after infestation in Ruston loamy-fine sand maintained under both the wet and wetting-drying regimes, and in autoclaved Astatula sand maintained under the wetting-drying regime. Propagule numbers in the Astatula sand under the wet regime decreased gradually between 7 and 90 dy after infestation.

Propagule counts were low initially and increased within 14 dy after the infestation of raw Astatula sand

with 5 or 250 chlamydospores of the fungus per gram of soil maintained at 25 C (Fig. 13, 14). The propagule numbers generally were lowest at both infestation levels in raw soils maintained in the wet regimes. Populations declined within 30 dy after infestation, and were low or undetectable 90 dy after infestation in raw soil at all moisture regimes at both initial infestation levels.

Although populations of the fungus in raw Astatula sand initially infested with 250 chlamydospores per gram of soil and maintained at 10 C did not increase over the initial number detected, except in the dry regime at 30 dy, the decline in the number of propagules was slower and the final propagule numbers at 90 dy were higher than in the same soil and moisture regimes at 25 C (Fig. 15). At 90 dy 13, 18, and 88 propagules were detected per gram of raw Astatula sand maintained at 10 C under the wet, wetting-drying, and dry regimes, respectively.

At the initial sampling 13, 7, and 13 propagules of the fungus were detected per gram of naturally infested, raw Ruston loamy-fine sand in the wet, wetting-drying, and dry moisture regimes at 25 C, respectively (Fig. 16). Although the populations of the fungus fluctuated throughout the duration of the experiment, the populations after 90 dy were about the same as those detected initially. When the raw Ruston loamy-fine sand

with residual populations of the fungus was maintained at 10 C, populations decreased to 2 propagules per gram of soil under all soil moisture regimes (Fig. 17).

In an additional survival study, low numbers of propagules of P. parasitica var. nicotianae (1-16 propagules per gram of soil) were detected after 8 mo in naturally infested, raw Ruston loamy-fine sand and autoclaved Ruston loamy-fine sand initially infested with 250 chlamydospores per gram of soil and maintained at 25 C. The fungus also was detected at 2 to 9 propagules per gram of soil of naturally infested, raw and artificially infested, autoclaved Ruston loamy-fine sand that had been maintained at 10 C for 12 mo.

Populations of P. parasitica var. nicotianae in the former root zones of plants in a tobacco field near Quincy, FL, were sampled monthly for 13 mo beginning 2 wk after the final leaf harvest in August 1977 and ending in September 1978 (Fig. 18). Points on the graph represent the mean populations determined from the former root zones of six susceptible Hicks plants or six moderately resistant Speight G-28 plants in each of two plots (designated A and B). At the first sampling date the highest population detected was 188 propagules of P. parasitica var. nicotianae per gram of soil from Hicks plants in plot A. Only 32 propagules of the fungus per gram of

soil were detected from the former root zones of Hicks plants in plot B. In August 1977, 146 and 101 propagules of the fungus per gram of soil were detected in soil around Speight plants in plot A and in plot B, respectively. Populations from around Hicks plants in plot A and Speight G-28 plants in plots A and B fluctuated throughout the sampling period, but the general trend was a reduction in the populations. Phytophthora parasitica var. nicotianae was detected in all plots 14 mo after the final harvest of 1977; 4, 0.3, 0.3, and 27 propagules of the fungus per gram of soil were detected in the former root zones of Hicks plants in plots A and B and in the former root zones of Speight G-28 plants in plots A and B, respectively.

Data presented in Fig. 19 are the means of populations detected in six to 12 random, individual samples from the whole field and from each of two plots designated C and D in the field near Quincy, FL. Two weeks after the final leaf harvest 12, 3, and 7.5 propagules of the fungus per gram of soil were detected in plot C, plot D, and in the random field sample, respectively. Populations decreased in all areas from September 1977 to December 1977 or January 1978 and then increased in March 1978 to 10 and 8 propagules of the fungus per gram of soil in plots C and D respectively. Two propagules of the fungus per

gram of soil were detected in samples from the whole field in March 1978. Phytophthora parasitica var. nicotianae was not detected in samples taken in April after the field soil had been disked, but it was detected again in all samples in May 1978. No further samples were taken from the whole field, but populations in plots C and D were 6 and 1 propagules of the fungus per gram of soil, respectively, in August 1978, 13 mo after the final harvest.

Phytophthora parasitica var. nicotianae was detected also in soil samples from the former root zones of tobacco plants and from random samples of a whole field in Gainesville, FL, from the end of the 1977 growing season through June 1978, when sampling ceased (Fig. 20). Mean populations of 68, 36, 31, and 6 propagules of the fungus per gram of soil were detected 2 wk after the final leaf harvest in samples from the former root zones of three Hicks, six Speight G-23, six Speight G-28 plants, and from the six random samples from the whole field, respectively. The populations from the former root zones of plants fluctuated throughout the sampling period, but decreases in the former root zones of resistant plants were not as rapid as in the former root zones of the Hicks plants. The population of *P. parasitica* var. *nicotianae* detected in random samples of the whole field declined to 0.3

propagule of the fungus per gram of soil in November 69
1977 and remained low, with 0.7 propagule of the fungus
per gram of soil detected in June 1978. Final populations
of 10, 2, and 5 propagules of the fungus per gram of soil
were detected in the former root zones of Hicks, Speight
G-23, and Speight G-28 plants, respectively.

TABLE 3. Populations of Phytophthora parasitica var. nicotinae and percent mortality of tobacco plants in autoclaved Astatula sand, raw Astatula sand, and autoclaved Ruston loamy-fine sand that had been infested with 5 chlamydospores of the fungus per gram of soil, or in raw Ruston loamy-fine sand with resident populations of the fungus. Soils were maintained at 25 C.

Soil treatment	Propagules ^a /g soil and % mortality ^b at days after soil infestation							
	0		1		4		7	
	no.	%	no.	%	no.	%	no.	%
Astatula sand								
autoclaved								
wet ^c	0	100	14	100	2	100	11	100
wetting-drying ^d	0	100	14	88	15	100	8	94
raw								
wet	1	100	19	100	2	100	11	58
wetting-drying	1	100	19	100	4	88	1	94
Ruston loamy-fine sand								
autoclaved								
wet	4	84	9	100	36	100	30	94
wetting-drying	4	100	9	100	39	100	35	100
raw ^e								
wet	13	67	13	100	9	100	6	94
wetting-drying	7	100	13	94	11	100	8	100

^a Propagules were detected by plating soil dilutions on a selective medium.

^b Percent mortality was determined 50 dy after susceptible tobacco plants were exposed to soil that had been maintained at 25 C for 0, 1, 4, or 7 dy.

^c The soil was maintained at a soil moisture content = 27% saturation.

^d The soil moisture content was allowed to fluctuate between 27 and 6.8% saturation.

^e Soil was collected from the root zones of plants infected with the fungus in a field near Quincy, FL.

Fig. 8. Populations of Phytophthora parasitica var. nicotianae at various days after autoclaved Ruston loamy-fine sand was infested with 250 chlamydospores of the fungus per gram of soil and maintained at 25 C under the wet (•—•), wetting-drying (o—o), and dry (x—x) soil moisture regimes.

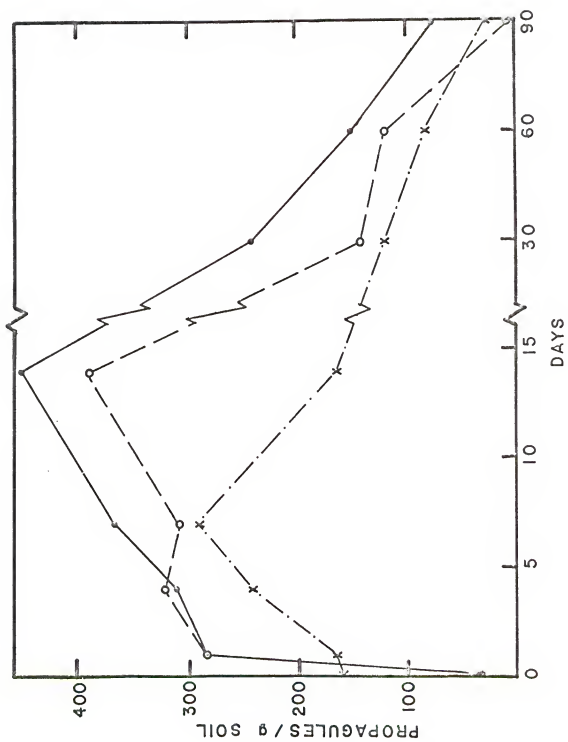


Fig. 9. Populations of Phytophthora parasitica var. nicotianae at various days after autoclaved Astatula sand was infested with 250 chlamydospores of the fungus per gram of soil and maintained at 25° C under the wet (—●—), wetting-drying (○—○), and dry (x--x) soil moisture regimes.

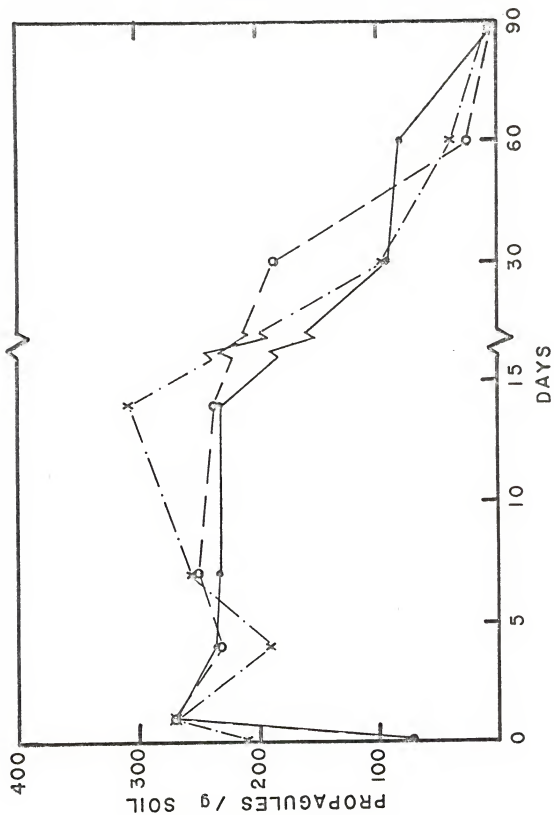


Fig. 10. Populations of *Phytophthora parasitica* var. *Nicotianae* at various days after autoclaved Ruston loamy-fine sand was infested with 250 chlamydospores of the fungus per gram of soil and maintained at 10 C under the wet (●—●), wetting-drying (○—○), and dry (x—x) soil moisture regimes.

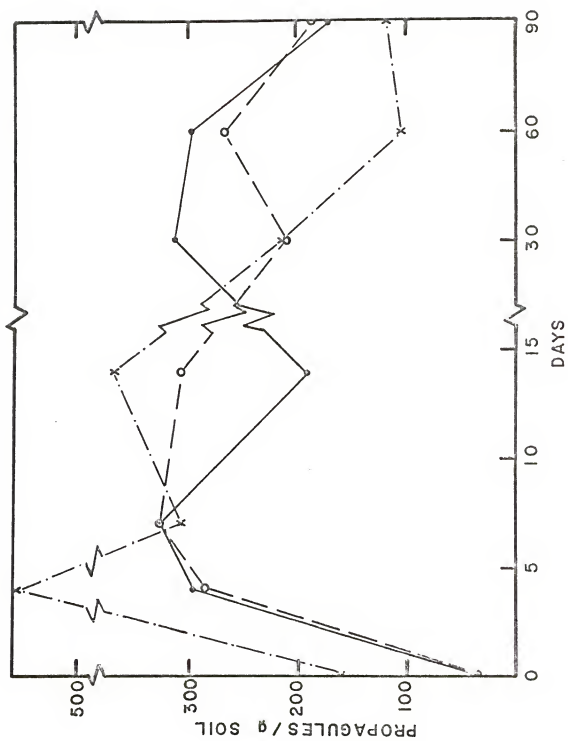


Fig. 11. Populations of Phytophthora parasitica var. nicotianae at various days after autoclaved Astatula sand was infested with 250 chlamydospores of the fungus per gram of soil and maintained at 10 C under the wet (●—●), wetting-drying (○—○), and dry (×—×) soil moisture regimes.

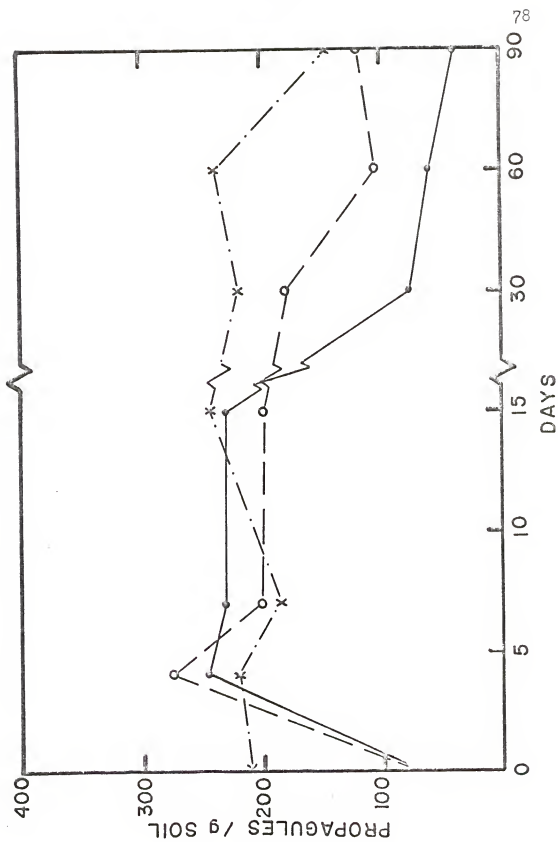


Fig. 12. Populations of Phytophthora parasitica var. nicotianae in autoclaved Astatula sand maintained at 25 C under the wet (●—●) and wetting-drying (○—○) soil moisture regimes, and in autoclaved Ruston loamy-fine sand at 25 C under the wet (x—x) and wetting-drying (Δ—Δ) soil moisture regimes at various days after infestation with 5 chlamydospores of the fungus per gram of soil.

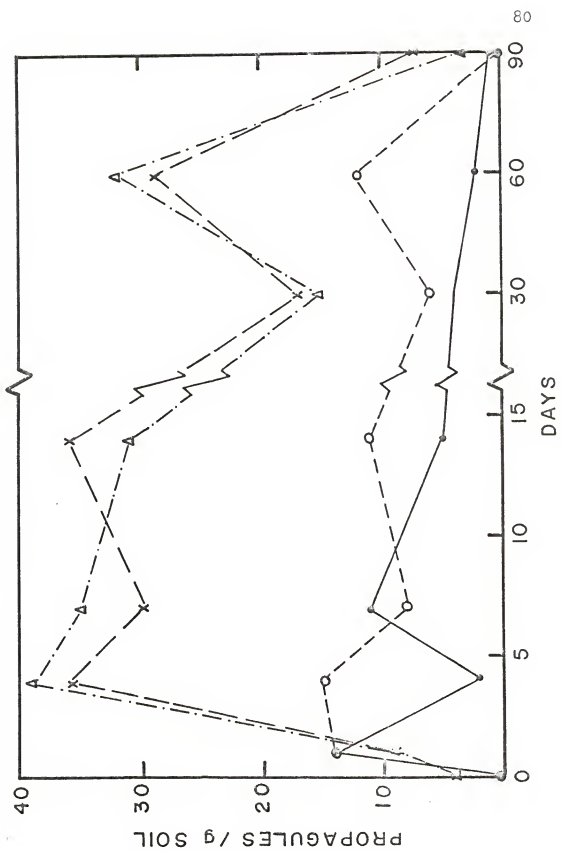


Fig. 13. Populations of *Phytophthora parasitica* var. *nicotianae* at various days after raw *Astatula* sand was infested with 5 chlamydospores of the fungus per gram of soil and maintained at 25 C under the wet (●—●) and wetting-drying (●—○) soil moisture regimes.

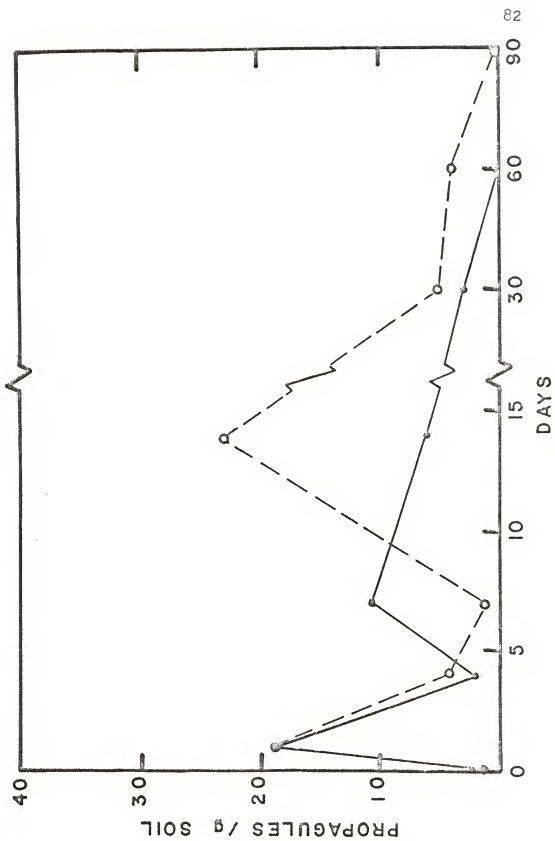


Fig. 14. Populations of *Phytophthora parasitica* var. *nicotianae* at various days after raw *Astatula* sand was infested with 250 chlamydospores of the fungus per gram of soil and maintained at 25 C under the wet (●—●), wetting-drying (○—○), and dry (×—×) soil moisture regimes.

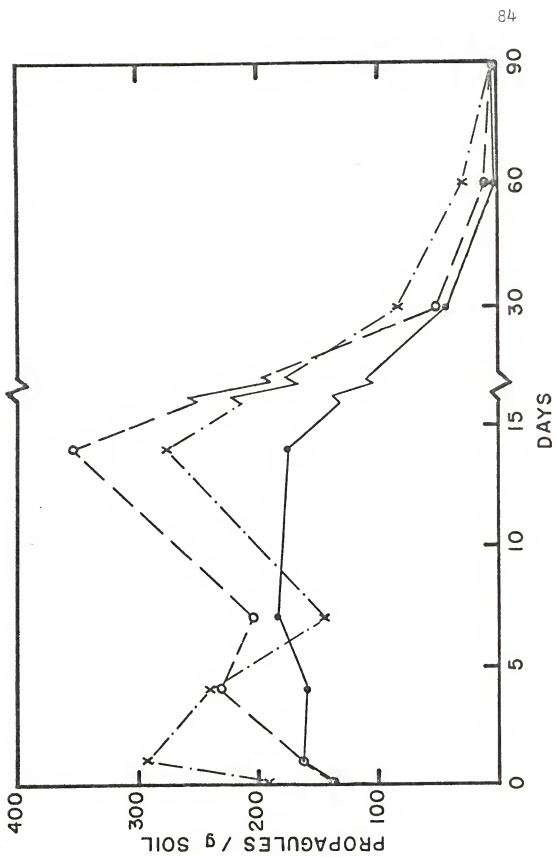


Fig. 15. Populations of Phytophthora parasitica var. nicotianae at various days after raw Astatula sand was infested with 250 chlamydospores of the fungus per gram of soil and maintained at 10 C under the wet (●—●), wetting-drying (○—○), and dry (x—x) soil moisture regimes.

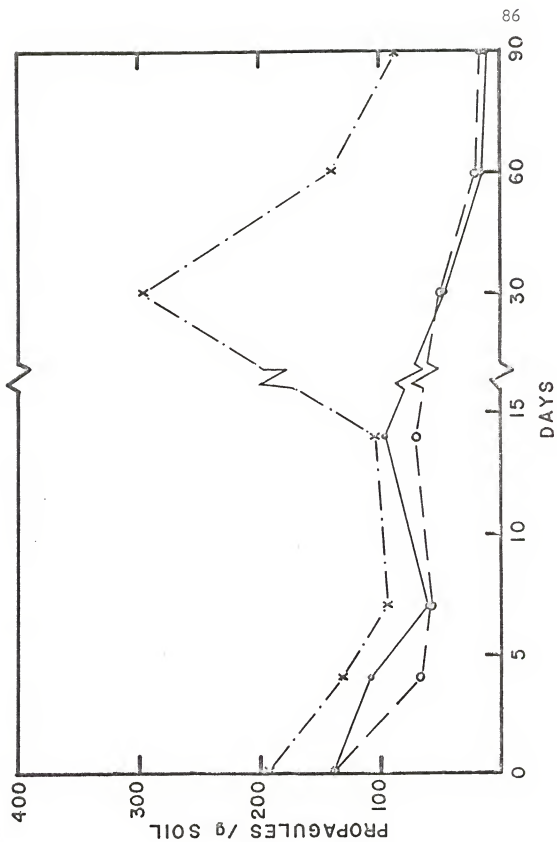


Fig. 16. Populations of Phytophthora parasitica var. nicotianae at various days after naturally infested, raw Ruston loamy-fine sand was maintained at 25 C under the wet (—●—), wetting-drying (○—○), and dry (x—x) soil moisture regimes.

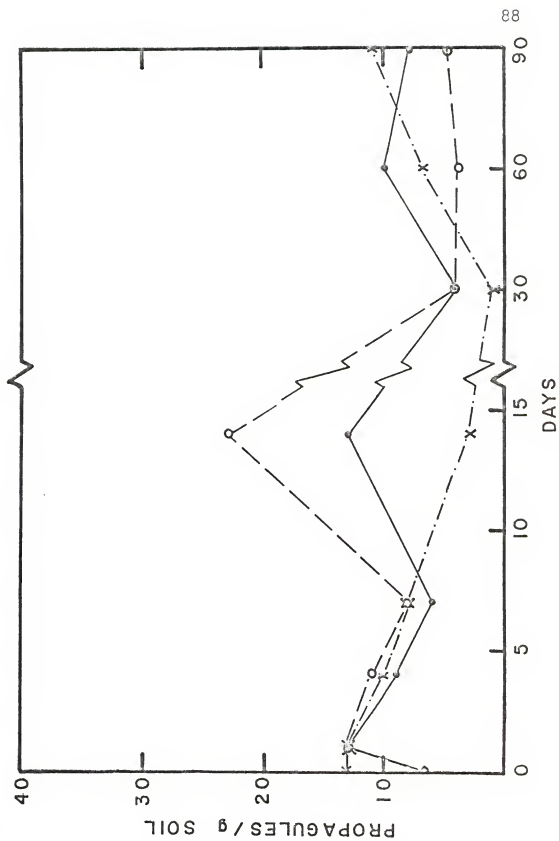


Fig. 17. Populations of Phytophthora parasitica
var. nicotianae at various days after naturally
infested, raw Ruston loamy-fine sand was maintained
at 10 C under the wet (●---●), wetting-drying
(○---○), and dry (x---x) soil moisture regimes.

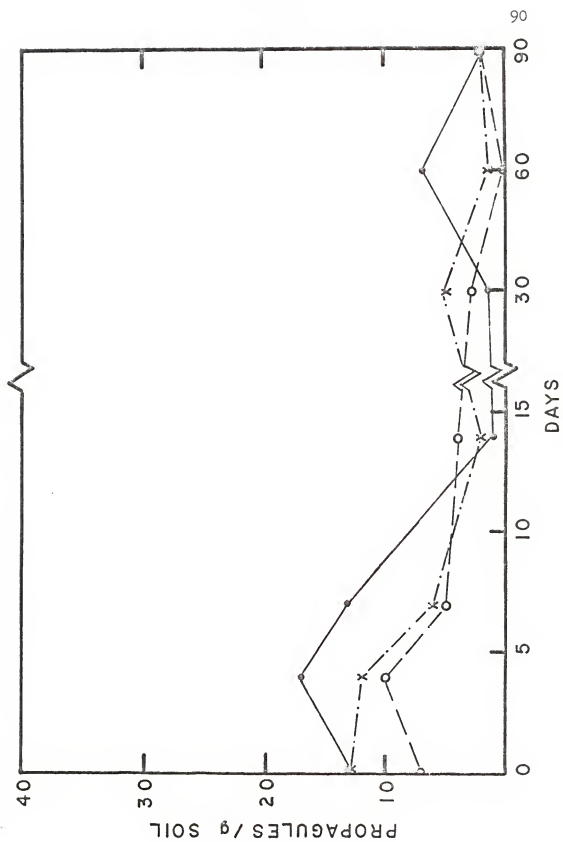


Fig. 18. Populations of *Phytophthora parasitica* var. *nicotianae* from August 1977 to September 1978 in the former root zones of Hicks (●—●) and Speight G-28 (○—○) tobacco plants in plot A, and Hicks (×—×) and Speight G-28 tobacco plants (Δ—Δ) in plot B (Quincy, FL). Values presented are the means of populations from six samples each.

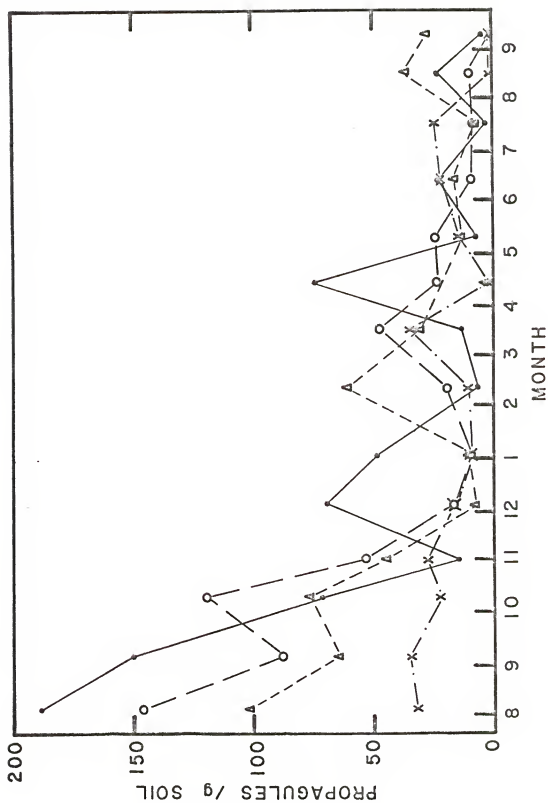


Fig. 19. Populations of Phytophthora parasitica var. nicotianae from random bulk samples of soil from plot C (●—●), plot D (x—x), and the mean of six bulk samples from the whole infested field near Quincy, FL (●—●) from August 1977 to September 1978.

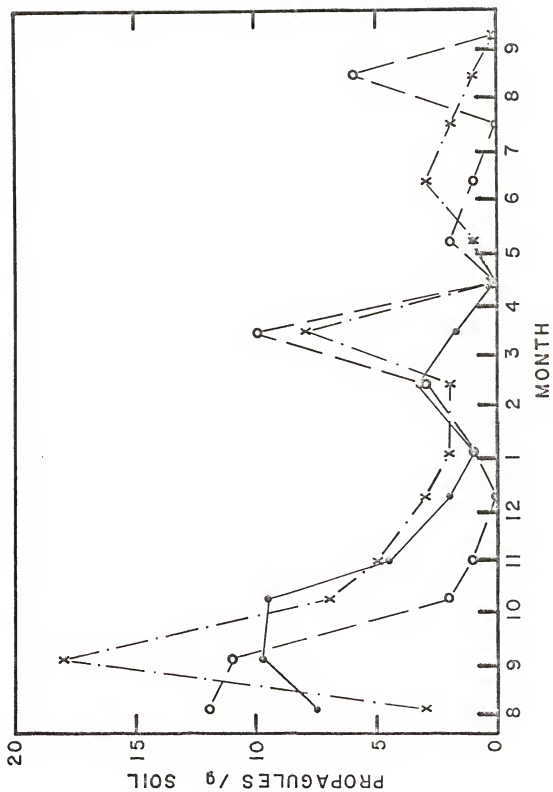
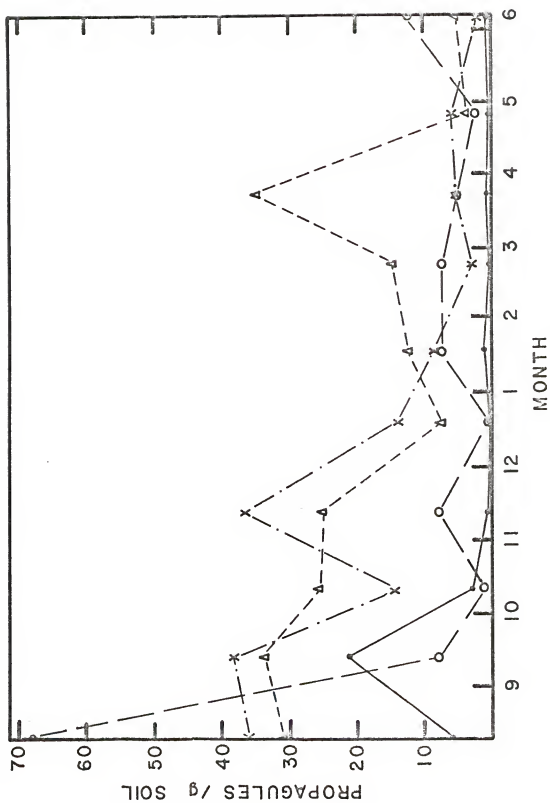


Fig. 20. Mean populations of Phytophthora parasitica var. Nicotianae in soil from the former root zones of three Hicks (6---9), six Speight G-23 (x--x), and six Speight G-28 (A--A) tobacco plants; and from six random bulk samples of the whole field (•---•) in Gainesville, FL, from August 1977 to June 1978.



The following trends were noted in laboratory studies on the effects of temperature and moisture on populations of P. parasitica var. nicotianae in soil: (i) fewer propagules of the fungus were detected immediately after infestation of soils than expected based on the number of chlamydospores incorporated into the soil, but the greatest initial recovery was from the dry soil treatments; (ii) the number of propagules detected increased within 2 wk after soil infestation, and the greatest increases occurred in autoclaved soils infested with 5 chlamydospores per gram of soil; (iii) propagule numbers decreased after 30 to 60 dy, but higher populations were maintained at 10 C than at 25 C after 90 dy in artificially infested soils; (iv) the number of propagules fluctuated less in naturally infested soil than in artificially infested soil; and (v) survival was greatest in the autoclaved Ruston loamy-fine sand than in autoclaved Astatula sand, and was poorest in raw Astatula sand.

Holdaway (16) made similar observations in studies on the effects of temperature and moisture on the survival of P. parasitica in soil. He found that the length of survival depended on the particular soil and was shortest in artificially infested raw soil. Survival of

P. parasitica also was better at 25% saturation than in dry soil or in wetter soils and was better at temperatures lower than 25 C (12 or 15 C). He suggested that increased survival at the lower temperatures may have been due to reduced rates of respiration of P. parasitica propagules at 12 or 15 C. Survival of P. parasitica var. nicotianae also was greater at a low temperature (10 C) than at 25 C. The reported minimal and optimal temperatures for the growth of this fungus are 10-12 and 25-30 C, respectively (26), and thus a reduction in active growth of P. parasitica var. nicotianae may have been responsible for the increased survival at 10 C. It is also possible that increased survival was due to reduced activity of organisms antagonistic to the Phytophthora spp., or perhaps the induction of chlamydospore germination was reduced at the lower temperatures.

Holdaway (16) reported that when sterile soil was infested with chlamydospores and maintained under axenic conditions at 50% saturation and 25 C, the populations of P. parasitica increased approximately 16-fold after 16 dy. Although bacterial contamination was detected after 20 dy, the population of P. parasitica after 50 dy was still nine times greater than detected initially. The population increase was attributed to the germination of the chlamydospores and subsequent production of secondary

chlamydospores and sporangia. Under the moisture conditions of his study it was concluded that population increases were not due to zoospore production since empty sporangia were seldom observed. Kelley (23) reported that Trichoderma spp. induced the formation of chlamydospores by P. cinnamomi in nonamended soil when the Trichoderma spp. were added to the soil at the same time as P. cinnamomi or 3 dy later. The production of chlamydospores was assumed to be associated with the depletion of nutrients in the soil (23, 24). It should be noted that P. cinnamomi has shown greater ability for saprophytic growth in soil than other Phytophthora spp. for which growth in soil has been examined (23, 24, 43, 50). The increases in populations of P. parasitica var. nicotianae reported in this study may reflect the germination of chlamydospores in response to nutrients in the soil, perhaps released by the autoclaving process, followed by the production of one or more germ tubes on which secondary chlamydospores and sporangia were formed. Autoclaved soils can be recolonized rapidly by saprophytic fungi, and in these studies the sporulation of Trichoderma spp. and other fungi occurred on the surface of the soil a few days after the soil was infested with P. parasitica var. nicotianae. It is possible that the depletion of soil nutrients by these fungi may have

influenced the observed increases in the populations of P. parasitica var. nicotianae. The possibility that the population increases were due to zoospore production and release is doubtful because soil moistures were unfavorable for zoospore release and precautions were taken in the plating procedures to minimize the time between placement of a sample in dilute agar and plating of the dilution.

The discrepancies between the initial number of chlamydospores mixed into the soil and the number of propagules that were detected on the selective medium may have been due to a shock to the spores, when they were placed in the soil, that resulted in low germination. The spores were viable because greater than 90% of the spores in the suspension used to infest the soil germinated on the selective medium, and because between 84 and 100% of the plants in the bioassay were killed after exposure to the infested soils for 50 dy at 25 C when between 0 and 4 propagules of the fungus per gram of soil were detected at the time of planting (TABLE 3). The low populations of P. parasitica var. nicotianae detected immediately after infestation of the soil, combined with changes in the detected populations within one to several days following soil infestation, reveal the possible errors that could result from the use of techniques to determine initial fungal populations based on the isolation of these fungi on selective media at some intervals

following soil infestation. These techniques also usually do not facilitate the determination of the types of propagules present. It is preferable, therefore, to quantify the inoculum prior to soil infestation or to use infested soil in which the populations of the fungi in question have stabilized.

The rapid decline of artificially established populations of Phytophthora spp. in raw soil may have been due to competition with well established micro-organisms for nutrients. The lysis of mycelium and other propagules produced by Phytophthora spp. in raw soil has been reported (29, 36, 43, 44). Holdaway (16) found that populations of P. parasitica were relatively stable in naturally infested raw soil. Flowers and Hendrix (11) found that naturally established populations of P. parasitica var. nicotianae in raw soil in pots maintained in a greenhouse or outside showed little fluctuation over 3 mo. Similar results were obtained in the laboratory experiments reported here. In these soils the Phytophthora spp. probably were adapted to some extent to the particular soil environment and to the biological interactions in the soil. Strict competition for nutrients and other proposed forms of fungistasis may have operated and inhibited the germination of fungal propagules in the absence of the host stimulation. As

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mentioned above, populations of P. parasitica var. nicotianae can survive in the field for at least 4 yr in the absence of host plants (1).

Flowers and Hendrix (11) monitored populations of the fungus in the rhizospheres of susceptible and resistant tobacco plants from the time of planting in the field (June) until the following March; they found that populations were low or undetectable initially, increased as the result of pathogenesis until the time the plants were killed (by the fungus or by frost), and then declined. They could not determine from their results whether the decline in the populations was in response to season or merely the result of the depletion of the rhizosphere soil from repeated sampling. Although high populations were detected in the former root zones of certain diseased plants throughout the sampling period, the fungus was rarely detected after the fields were plowed. In the present study populations of P. parasitica var. nicotianae also were high in soil around the roots of diseased or dead plants following the growing season and, as reported by Flowers and Hendrix (11), population levels appeared to be related to the amount and rate of disease development. Mortality of tobacco plants was greater and root-zone populations of P. parasitica var. nicotianae 2 wk after harvest generally were higher with susceptible

plants than with resistant plants in both fields. Populations around the former root zones of Hicks plants in plot B in Quincy were low 2 wk after harvest (Fig. 18). Since the maximum populations of the fungus occurred around the time of plant death (11, 20) and the plants in that plot died early in the season, it is likely that populations of the fungus had declined by the time of the post-harvest sample. Although populations of P. parasitica var. nicotianae in the former root zones generally decreased with time, they still were detected 10 mo after harvest in the field in Gainesville and 14 mo after harvest in the field near Quincy. Populations of the fungus determined from random samples of the two fields, which included alleys as well as former rows of plants, and from plots C and D, which formerly contained rows of plants, were high in the Quincy field in September. The decline in populations in both fields probably was mainly due to the dispersal of propagules when the field was plowed in late September, but may have been due also to the death of less resistant propagules caused by reductions in soil moisture and temperature that occurred around the same time. Between 0.2 and 0.7 propagule of P. parasitica var. nicotianae per gram of soil was detected during the remainder of the sampling time. Populations of the fungus also declined during

the fall and winter in the field near Quincy. The following spring the populations increased, perhaps due to an increase in germination of propagules of the fungus and production of secondary propagules in response to increases in soil moisture and temperature, but only 0.75 propagule of the fungus per gram of soil was detected in samples taken a few days after soil was plowed in mid-April. The populations increased again at a later sampling date.

Although populations of *P. parasitica* var. *nicotianae* declined rapidly in the field after the growing season, there appeared to be residual propagules that were able to survive for long periods of time (11, 12, 41). Chlamydo spores and fungal propagules in dead plant tissue were identified as the origins of colonies on the selective medium. Flowers and Hendrix (10) also found that chlamydo spores were the primary survival structures isolated from the soil. Since chlamydo spores are the primary residual propagules of this fungus in soil, and thus serve as the primary source of initial inoculum, they should be utilized more often in studies designed to simulate aspects of the host-pathogen interaction in the field. There are no known reports of the utilization of chlamydo spores in studies on genetic (and pathogenic) variability of this fungus, and few reports where

chlamydospores have been utilized as the sole source of inoculum in studies of plant infection (20, 21, 33).

The ability to detect and quantify inoculum of a pathogen is important in attempts to forecast the occurrence and severity of many plant diseases. Based on the results of this study, it is suggested that the following points be considered in future attempts to determine initial populations of P. parasitica var. nicotianae in the field: (i) take samples from the area of former plant rows; (ii) sample when the soil is moist, i.e., a few days after a rain or irrigation, because the detection of Phytophthora spp. is often greater after dry soil has been remoistened (16); (iii) sample before or several days after the soil is plowed as the detection of the fungus generally is reduced immediately after the soil is plowed; and (iv) use a selective medium that allows identification of the fungus from macroscopic observations without confusion with other fungi or interference from faster growing fungi such as species of Pythium.

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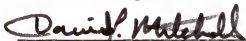
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BIOGRAPHICAL SKETCH

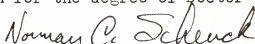
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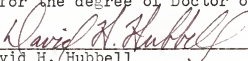
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March, 1979



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